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NUTRITIONAL ASPECTS OF BONE HEALTH AND MUSCLE PERFORMANCE IN THE ELDERLY IN THE UK

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ABSTRACT

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DIETARY ASPECTS OF BONE HEALTH AND MUSCLE PERFORMANCE IN THE ELDERLY IN THE UK

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To date, studies of diet and osteoporosis have mainly focused on single nutrients with few studies assessing more than calcium, protein and vitamin D intake. Because of the complex interaction between nutrients, attention has moved toward exploring overall dietary patterns. This study tests the hypothesis that a diet, which complies with current healthy dietary guidelines, is associated with lower plasma alkaline phosphatase (ALP) and stronger handgrip strength as markers of bone health and muscle performance in older people. The study also examines whether men, and those with optimal early nutrition will benefit more from a healthy diet than women and those from a less than optimal early environment. This is the first study to examine the effect of a healthy dietary pattern and to explore its interactions with sex and body size measurements on bone health and muscle performance in the elderly.

The secondary analyses undertaken in this study are based on the data of the National Diet and Nutrition Survey (NDNS) conducted on a UK nationally-representative sample of 1687 men and women aged 65 years and over. Principal component analysis (PCA) was used to summarise dietary patterns, by which seven statistically independent eating patterns were generated. Subject's factor loading scores were derived for each eating pattern. Correlations between these dietary scores and alkaline phosphatase (ALP) and handgrip strength were examined and individuals were categorized according to their dietary pattern.

The healthy dietary pattern identified by PCA characterised by a high intake of vegetables, fruits, cereals, fish and other seafood, showed the strongest negative (beneficial) association with ALP ($r = -0.17$, $p<0.001$) and the strongest positive association with handgrip strength ($r = 0.29$, $p<0.001$). Multiple regression analysis controlling for energy intake, a number of confounders and various nutrients, identified the healthy diet as the strongest predictor for serum ALP and handgrip strength in elderly men and women, separately. Subjects in the highest fourth of the healthy diet in comparison to the lowest, were less likely to have high levels of plasma ALP (OR = 0.4, 95% CI, 0.3 – 0.6) after adjustment for known confounders. Healthy diet was of the most benefit for heaviest and tallest men but not heavier and taller women. For those within the shortest and thinnest group, the association between healthy diet and ALP did not reach statistical significance. According to the most frequently practiced eating pattern, only 2% of people from institutions and 14% of community dwelling people in the UK could be considered as healthy eaters.

The overall dietary pattern may be more important in predicting bone health and muscle performance in the elderly than any single nutrient. An eating pattern, which complies with the current healthy dietary guidelines in the UK may be associated with good bone health and better muscle performance. These results suggest the need to improve the eating habits of the elderly population in the UK. These results have implications for preventive programs aimed at improving and maintaining bone health and muscle performance in the elderly by focusing on the entire diet as a comprehensive approach, rather than just calcium and vitamin D.

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Abbreviations and special term definitions

Abbreviations:

B ALP	- Bone Alkaline Phosphatase
T ALP	-Total Alkaline Phosphatase
BMI	-Body Mass Index [weight (kg)/height $(m)^2$]
COMA	-Committee on Medical Aspects of Food and Nutrition Policy
DBP	-Vitamin D Binding Protein
DXA	-Dual X-ray Absorptiometry
GH	-Growth Hormone
IGF	-Insulin-like Growth Factor
LEP	-Leg Extension Power
OC	-Osteocalcin
PEM	-Protein Energy Malnutrition
PTH	-Parathyroid Hormone
SM	-Skeletal Muscle Mass
VDR	-Vitamin D Receptor

Special term definitions:

BMC -Bone Mineral Content: The mass of bone mineral in a skeletal unit (g) or a certain length (g/cm).

BMD -Bone Mineral Density: The density of bone in a skeletal unit (g/cm^3) or in an area of the scanned bone envelope (g/cm^2).It is not a true density measurement.

RDA -Recommended Dietary Allowance (a term in use in many countries such as USA, Canada and Asian nations but not in the UK).

RDA -Recommended Daily Amount of food, energy and nutrient: this term had been used previously in the UK (1979).

DRVs -Dietary Reference values. This term is used for LRNI,EAR,RNI and safe intake and is recommended by COMA.

EAR -"Estimated Average Requirement of a group of people for energy or protein or a vitamin or mineral. About half will usually need more than EAR, and half less"(509).

RNI -Reference Nutrient Index; that is a value of 2 standard deviations above the EAR. This amount of nutrients is enough or more than enough for about 97.5% of people in a group. If average intake of a population is at the RNI, the risk of deficiency is small(509).

LRNI -Lower reference nutrient intake. An amount of the nutrient that is enough only for a few people in the group who have low needs(509).

BMR	-Basal Metabolic Rate: Rate at which the body uses energy when the body is at complete rest.
Safe intake	-An amount that is enough for almost everyone but not so large as to cause undesirable effects. This term is used when there is not enough information to estimate DRVs.

1. Introduction

1.1. Osteoporosis, definition and clinical manifestations

By definition, osteoporosis is a systemic chronic disorder of the skeleton characterized by low bone mass and micro-architectural deterioration of bone tissue. It is the commonest metabolic disease of bone, in which the composition of bone is essentially normal but the amount of bone mass is decreased throughout the body (4;5). The definition proposed by WHO is: "A disease characterized by low bone mass and micro-architectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk" (4). The quantitative definition of osteoporosis using bone mineral status is: "A value for BMC (Bone Mineral Content) or BMD (Bone Mineral density) 2.5 standard deviations or more below the mean for young adults" (4). Bone measurements in this definition are measures of the spine, hip and forearm. Although these definitions are practically helpful, they face some limitations, which are important when epidemiological evidence is reviewed; the first definition has not defined a threshold level or cut-off point for architectural deterioration and the second has not been included the concept of bone structure, which is obviously important for bone strength. Bone mass is only one factor in predicting the risk of osteoporotic fractures, the bone structure is the other one, which could not be measured by bone mineral status. It is also important to note that the WHO criteria refer only to mineral measurements of the spine, hip, or forearm (6) and, therefore, may not be perfect in predicting the bone measurements at other sites, although bone mineral status at different skeletal sites are highly inter-correlated. Furthermore, BMC and BMD are influenced by body size. Thus, these may not be proper for comparing populations with different body sizes.

However, low bone mass measured by BMC or BMD is a risk factor for fragility fractures (6-9). Significant association between low bone mass and increased osteoporotic fracture risk have been documented in both cross-sectional and prospective studies (10-14). The risk of nonspine fractures has been shown to increase about 1.6 fold by each SD decline in bone density (15) and the risk of vertebral fractures has been shown to increase 2.0-2.4 fold for each SD decrease in bone density (16).

Histologically, osteoporotic bone tissues have fewer, thinner, and less connecting trabeculae in comparison with normal bones. The overall result is a decrease in bone strength and, therefore, a high susceptibility to fracture, a common feature of the osteoporosis (17).

Osteoporosis can be classified as type I (post menopausal) and type II (senile), the former appears in the years immediately after menopause and the latter is found in people of advanced age. Type I occurs between ages 50-75 years and trabecular bone fractures (vertebral and the distal radius) are more common because trabecular bones are more involved than cortical bones. Oestrogen deficiency is recognised as the most important pathogenetic factor of type I osteoporosis and women are approximately affected six times as men. In type II osteoporosis, on the other hand, bone involvement is more balanced for trabecular and cortical bones (18;19). This type of osteoporosis affects men and women over the age of 70 years (women: men 2:1, approximately) and fractures are commonly seen in the proximal femur (hip) and the spinal vertebrae (wedge fracture). Many fractures may remain asymptomatic, especially compressive fractures of the spine, and thus, may not become clinically attended.

1.2. Osteoporosis; epidemiology and trends

Osteoporosis is a worldwide public health problem that is growing in importance as the population ages (1;2). In 1992 about 1.7 million hip fracture occurred across the world, from which more than half happened in the Europe (1;22). Currently, the incidence of hip fracture is thought to be about 1.66 million per year across the world, and it has been projected that the number of hip fractures worldwide will increase to 6.26 million by 2050 (24). If secular trend is taken to account, this number could range between 7.3 and 21.3 million by 2050 (25).

It has been estimated that approximately 30% of postmenopausal white women suffer from osteoporosis in the Unites Ststes (20). Another estimation by from US, Riggs et al indicates that the lifetime risk of osteoporotic fractures is 13% in men and 40% in women after the age of 50 (22). Jacobsen et al (23) have reported a rate of 35.4/1000 per year for hip fractures among women aged 95 years in the United States. They also estimated that about 200,000 hip fractures occur in the United States each year. Approximately, 1.5 mil-

lion fractures annually are attributable to the osteoporosis in the USA (22). It has been estimated that one in three women and one in twelve men in the UK are affected by osteoporosis (36). Currently more than three million people are suffering from osteoporotic fractures in the UK. 60,000 hip fractures, 50,000 wrist fractures and 40,000 vertebral fractures occur each year in the UK (36).

Although, it is generally regarded as a disorder of postmenopausal women, osteoporosis is a major health problem for men in advanced ages (20;23;26-28); about 30% of hip fractures and 20% of vertebral fractures occur in men (24;29).

The risk of osteoporotic fractures increases exponentially with age (30), and, therefore, raising the proportion of older people due to expanding of life expectancy may increase the number of fractured patients, dramatically (22). In addition, there is a secular increase in the incidence of fractures, particularly in industrialized countries (2;31;32). Age-specific incidence of osteoporotic fractures, has increased over recent decades (26;33); though some reports show that this trend has begun to level off (34). The reasons for this secular trend are uncertain. A number of suggestions have been made, including lower physical activity due to different labor-saving devices, which are widely available in developed countries and is growing in developing countries (33), changes in dietary intakes, increasing in alcohol consumption, smoking and using sedatives (33;34). A cohort effect with persons who suffered from dietary restrictions during World War II has also been suggested (33;35), which is believed to affect the peak bone mass during the growth. However, rapid increase in the number of fractured patients implies the need for preventive strategies to be taken now to stop this catastrophic health problem.

The economic costs of osteoporotic fractures are difficult to determine, but in the UK estimated an annual expenditure of £742 million (36). It has been estimated that in western nations hip fracture costs about eight to ten million U.S. dollars per million population each year (37). In the United States the modest projected estimation of economic cost for only hip fractures in 2040 is over $2 billion (38).

The main complication of osteoporosis is an increased risk of fractures. In fact, osteoporosis is an asymptomatic condition until a fracture occurs. In rare circumstances other

complications may cause clinical manifestations, such as organ malfunctions due to body deformations. Only osteoporotic hip fractures are associated with mortality in up to 20% of cases within a year (2). Because osteoporotic fractures have such a devastating impact on the economics of medical care, particularly in developing countries, reducing the incidence of fractures by preventing osteoporosis is of particular importance.

1.3. Risk factors

Osteoporosis develops over many years and is the result of the cumulative effect of many factors including both genetic and environmental. In fact, low bone mass in the elderly may be a manifestation of factors influencing the skeleton in early life, even in infancy and childhood as well as adolescence and elderly ages (39-42). Bone mass in the elderly is determined by the maximum density and strength achieved at maturity (peak bone mass) and the amount of bone lost during aging (35). Therefore, low bone mass (osteoporosis) might be a result of sub-optimal bone gain in young adulthood (peak bone mass), bone loss in later life, or both.

Peak bone mass can involve both an increase in volume density and an increase in bone size. The skeleton will grow in length, in breadth, and in mass as the body grows but the consolidation of skeletal mass continues for some time after the the fusion of the epiphysal plates in long bones and cessation of growth in skeletal length. By an ideal environment (efficient nutrient supply and proper lifestyle) acquisition of bone mass will achieve its full genetical potential for bone mass and reaches to a plateau. 60-80% of variation in the peak bone mass is accounted for by genetic influences (451), thus a substantial proportion is still related to environmental and lifestyle influences during childhood, such as diet and physical activity. The genetical influence on peak bone mass is polygenic in nature and the involved genes have not been specifically identified. Most important environmental influences in this regard are nutrition (protein, calcium, vitamins C, D and K, minerals copper, manganese, zinc and phosphorus) and physical activity. The age at which total skeletal mass peaks varies with skeletal site and with the population under study and the assessment of bone density mostly is confounded by the skeletal size. Peak bone mass in midlife is a major factor in determination of subsequent fracture risk and therefore any adverse environmental effect, such as insufficient nutrient supply, smoking,

alcohol drinking and/or suboptimal mechanical loading (physical activity) can pose a threat on the bone health in the elderly (285). After peak bone mass, there is a slow decline in bone mineral mass, which can be accelerated by age and different bone-affecting factors and may be resulted in osteoporosis and different osteoporotic fractures in old ages.

Within the context of this thesis, the association between diet and bone health in the elderly will be examined. Therefore, it is necessary to examine the literature and evidence of the role diet, in terms of foods or nutrients, in bone health, muscle function and fracture risk in older people. However, it is also necessary to understand the role of other constitutional, behavioural, and hormonal factors that may affect bone metabolism. As stated above, osteoporosis is a multifactorial disorder with numerous aetiologies (21). Furthermore, fracture risk increases as a function of poor bone health and factors influencing the propensity to fall. There is a complex mix of factors that affect bone health and risk of falling. These factors may be grouped as constitutional (age, gender, ethnic group) (21) or behavioural (alcohol intake, smoking habits, diet and activity), which may affect bone metabolism and muscle function (2;21;43-50). The interplay between these factors is complex and at present poorly understood. Among these factors nutritional factors may be of particular importance, even though they could not account for the totality of the problem. Physiologically, most nutrients influence bone metabolism but their importance in the pathology of osteoporosis varies from one nutrient to another (see chapter two) and may be affected by each other. Nutritional factors are also important to determine the risk of fall and fall-related trauma. Considering both physiologic bases and epidemiologic evidence in this regard may provide valuable information needed for preventive strategies to reduce this growing problem.

To date, the extent to which diet may affect bone health and risk of falling and thereby, risk of fracture in the elderly is unclear. To date, most attention has focused on a number of specific nutrients (mainly calcium; vitamin D, protein) studied in relative isolation and conflicting results have been reported. The main limitation of the studies on diet and bone health that can lead to discrepant results is that they considered some aspects of diet and neglected other aspects. Strong correlations and interactions among different nutrients

make it difficult to examine their effects separately. Therefore, to encapsulate effects of different nutrients and non-nutrients on bone health and muscle performance, pattern analysis was used in this study to characterise dietary patterns in a population and examine their associations with bone health and muscle function. Such analyses are food based rather than nutrient, and therefore, will investigate whether a certain combination of food (eating pattern) can affect bone metabolism and muscle performance in a positive or negative way. However, there are national food guidelines for the general population in the UK (448), which are food-based and thus it is important to verify the association between a diet, which complies with these guidelines, and bone health in people most at risk for osteoporotic fractures.

Hence, this study is primarily aimed to determine whether a diet, which complies generally with the national guidelines in the UK, is associated with lower plasma ALP and stronger muscle performance in older people. Such a pattern will be one of several dietary patterns generated by principal component analysis from data of dietary intakes, and will be the most compatible diet with the guidelines. It will come out from the data rather than being predefined, and therefore, in reality, will indicate how different foods may consume together and come to the diet.

The aim of this study is to explore the effects of dietary patterns on risk of poor bone health and muscle function. Alkaline phosphatase (ALP) will be used as a marker of bone health and handgrip strength will be used as a marker of muscle function

1.4. Hypotheses

The study hypotheses are stated below:

Primary hypothesis: Older people who eat a diet that complies with the healthy dietary guidelines have lower ALP and stronger handgrip strength than those eating a less healthy diet.

Secondary hypothesis: The effect of the healthy diet on bone health is greater in those who were optimally nourished in early life (using body size as a proxy measure).

Tertiary hypothesis: The relationships between current diet, early nutrition with bone health and handgrip strength will be stronger in men than women.

1.5. Aim

To determine whether a diet that complies with the healthy eating guidelines is associated with lower ALP and stronger grip strength in the elderly.

1.6. Objectives:

In order to address the study hypotheses following objectives were considered:

For the primary hypothesis, objectives were:

1. To characterise eating behaviours and their determinants among the elderly population in the UK.
2. To explore the relationship between dietary patterns and plasma ALP as a marker of bone health.
3. To determine the relationship between dietary patterns and handgrip strength as a marker of muscle performance in older people.

For the secondary hypothesis, the objective was:

- To determine the effect of body size on the relationships between dietary patterns, ALP and handgrip strength.

For the tertiary hypothesis, the objective was:

To explore hypotheses one and two in gender specific analyses; the assumption being that hormonal factors will play greater part in variation in risk in women than men.

1.7. Assumptions of the study

- Dietary patterns are more informative about risk of poor nutrition (nutritional status) related to bone health than individual nutrients studied in isolation.
- Plasma ALP is a marker of bone health; higher levels are associated with osteoporosis and therefore indicate poorer bone health.
- Handgrip strength is a marker of muscle performance: lower values are associated with poorer muscle performance.
- Body size, measured by weight and height, is a marker of early nutrition.

For the purpose of the study, within a limited timeframe of a PhD and the funding, it was decided to use dietary data on a population-base nutritional information. National Diet and Nutritional Survey of people aged 65 years or over in mainland Britain (NDNS)(59)

was used for analyses presented in this thesis, because of high quality of dietary data (4-day weighted dietary record, coding about 3500 food items), providing valuable information on a wide range of variables, which are important for bone health, such as smoking, drinking, physical activity, illness, anthropometry, different blood analytes and being a representative sample of people most at risk for osteoporotic fractures in the UK.

1.8. Implications of the study

The results of this study would be of benefit:

- To inform future research on bone health and nutrition.
- To consider the implications for preventive programs aimed at improving and maintaining bone health in the elderly.

1.9. Layout of the thesis

This thesis is organised into eight chapters: The first chapter gives an overview of the research and its aims and objectives. The second chapter reviews various aspects of the published literature on bone health and muscle function, with more emphases placed on nutritional factors. Chapters three through seven present analyses undertaken in this thesis using data from the National Diet and Nutrition Survey. Chapter eight discusses the results and draws conclusions. Specifically:

- Chapter one presents an overview of the study, states the hypotheses, aims, objectives and assumptions of the study to introduce the thesis.
- Chapter two briefly describes the physiology and metabolism of bone and presents a critical review of published literature of bone health and muscle function.
- Chapter three describes sample selection, data collection and procedures of NDNS from which data were used in this thesis. This chapter also describes the methodology of the study.
- Chapter four presents principal component analysis, dietary patterns and their relations to some characteristics of the population under study, which may affect bone health in the elderly in the UK. This chapter is mainly concerned about the first objective of the study (of the primary hypothesis), mentioned above.

- Chapter five explores the relationship between diet and plasma ALP in the elderly. Associations between dietary patterns and alkaline phosphatase are determined while the effects of other bone-affecting variables are controlled for. The second objective of the primary hypothesis will be addressed in this chapter.
- Chapter six describes the effect of body size on the relationships between diet and ALP. These relations are evaluated by a stratified analysis among men and women, separately. The effects of body weight and height on these relations are investigated after controlling for possible confounders.
- Chapter seven concerns about associations between dietary patterns and muscle performance, evaluated by handgrip strength, using multiple regression analysis and controlling for a number of confounders.
- Chapter eight is a discussion of the overall results. Comparison to other studies, presenting the possible explanations for the findings and conclusions are discussed in this chapter. It also presents the implications of the study and future work.

2. Literature review

The purpose of this section is to critically review the published literature on bone health and muscle performance, to build and develop the theoretical propositions. This review will attempt to critically appraise all the relevant epidemiological, clinical and laboratorial studies. The focus of this literature review is the role of nutrition in bone health and muscle function in the elderly. However, it is necessary to understand the role of other factors that may affect bone health and muscle function. On their own, none of factors will determine bone health in total, but their effects must be taken to consideration when analyzing how diet may be associated with osteoporotic fractures.

This literature review may be considered as having three sections. The first of these will present a brief description of bone physiology and metabolism. The second section of the literature review deals with how bone health may be affected by various nutritional and non-nutritional factors in the elderly and how it may be monitored by biomarkers. It is acknowledged that bone health in older ages is determined by the maximum density and strength achieved at maturity (peak bone mass) and the amount of bone lost during advancing age. Therefore, the effects of nutrition in the elderly may only be a part of the picture. Examining the evidence of the role of different variables on peak bone mass is out of the scope of this thesis

The third section will examine the literature and evidence of the effects of various factors on muscle function in the elderly.

In each section, studies of similar methodology will be considered together and their results will be appraised and compared. For the sake of saving the space, where necessary or helpful, the layout of the similar studies is tabulated and their specific points are discussed in the text.

2.1. The physiology of bone

Within the context of this section, only key aspects of bone physiology, which may be related to bone metabolism and is necessary for understanding the biologic relevance of

factors affecting bone health, will be presented. Full details of histology and physiology of bone and the pathophysiology of bone health are presented in related textbooks (17;51).

2.1.1. Bone tissue elements

There are three types of bone: compact, trabecular and woven bones. Compact bone is mainly found in the shaft of long bones. Trabecular bone is found in the vertebrae, the ends of the long bones and flat bones. Woven bone is an immature bone tissue found at fracture sites and will be replaced by one the two other types.

Cellular elements of bone tissue are osteoblasts, osteocytes and osteoclasts. The function of osteoblasts is to lay down osteoid by synthesis of collagen and glycoproteins, concentration of certain plasma proteins in matrix and maintenance of mineral homeostasis. Osteoblasts also play important roles in the mechanism of calcification. The function of osteoblasts is influenced by many factors such as vitamin D metabolites, thyroid, IGF-I and IGF-II, parathyroid, and sex hormones (Figure-2.1) (17). Local factors such as cytokines and genetic factors as well as mechanical factors are also important in their function (53).

The function of the osteocytes is still controversial. They are thought to play roles for the maintenance of bone matrix. The young osteocytes directly contribute to the formation of bone matrix. They have receptors for oestrogen and progesterone hormones by which their bone-forming activities can be stimulated. Mechanical loading and the function of muscle are also major stimulators of osteocytes. Under the influence of exercise, mechanical loading and gravity, osteocytes produce nitric oxide (NO) and prostaglandins (PG) E_2, both of which are responsible for osteogenesis by modifying the activity of osteoblasts and osteoclasts. Osteocytes are associated with osteoblasts in maintaining a constant minute-to-minute level of plasma calcium and inorganic phosphate between bone and tissue fluid. This movement of ions is thought to be controlled by PTH (17).

The function of the osteoclasts is to erode and resorb previously formed bone. Osteoclast can also freshly synthesise collagenase, on demand, without storing it. PTH increases the number and the activity of osteoclasts and vitamin D increases their activity without any effect on the number of osteoclasts (17). Osteoclastic activities may be mediated via osteoblasts (Figure-2.1) by production of a variety of osteoclast-sensitive cytokines (52;53).

Bone matrix is mainly composed of collagen, but some other non-collagenic compounds also exist in bone matrix. Five types of collagen molecules are recognized and the most important one in adult bones is type I collagen, which is also the major structural protein in tendons and skin and is synthesised by osteoblasts. It has a triple-helical structure containing two identical $\alpha_1$chain and one structurally identical but genetically different α_2 chain (52).These chains are coiled in a left-handed helix with about 3 amino acids per turn. Collagen is first synthesised in osteoblast in the form of a longer molecule, procollagen, that contains additional propeptides at both ends of its polypeptide chains. It is then extruded to the matrix for further processing, conversion to the collagen, by assembling of the fibrils into the fibres. This extra cellular conversion process requires at least two enzymes, a procollagen aminoprotease that removes the amino propeptides, and a procollagen carboxyprotease that removes the carboxy propeptides. Collagen molecules produced by cleavage of procollagen spontaneously assemble into fibrils that are microscopically indistinguishable from the mature fibrils found in tissues.

Stability of chains and the helical structure of collagen molecule are important for bone strength and are depended upon the existence of enough hydroxy proline and hydroxy lysine. Vitamin C and ferrous iron are required as a co-factor for the hydroxylation of these two amino acids. Copper is also necessary for lysine hydroxylation (17;51). Collagen forms a basis for mineralization and its circulating degradation's products have been used as markers for bone turnover and bone resorption.

Minerals in bone are mostly in the form of hydroxy apatite crystals by the formula of $Ca_{10}(PO_4)_6OH_2$. Carbonate is the third-most- abundant ion in bone mineral and constitutes about 5 percent of the total weight of ashed bone. Traces of iron, copper, lead, manganese, tin, aluminium, strontium and boron have also been detected in bone(17)

2.1.2. Physiology and metabolism of bone

2.1.2.1. Functions of the skeleton

The main functions of the skeleton are to protect the tissues of the body (e.g. the brain, the lung and pelvic tissues), supporting body organs and providing a framework to enable body movements, providing a huge source of blood calcium and buffer elements.

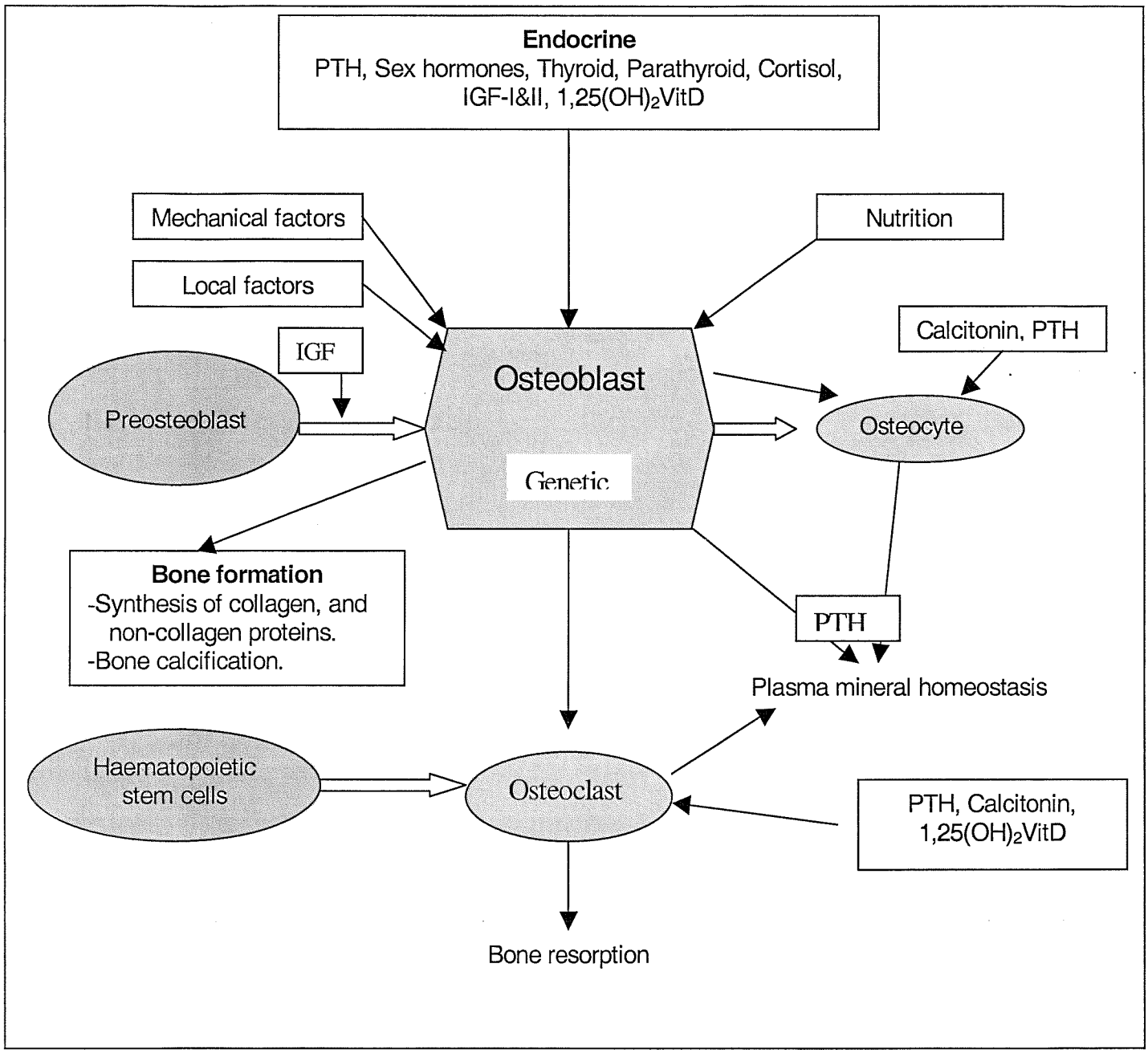


Figure 2. 1- Bone metabolism and the central role of osteoblast.

The two types of bone play different roles in the body; trabecular (cancellous) bones mainly are important in metabolic activities, haematopoiesis and plasma mineral homeostasis, while compact bones are more important in mechanical activities such as protection of vital organs, providing a support for body movements and weight bearing activities. Differences in the function and structures of these two types of bone tissue provide a physiological explanation for great variation seen in composition of different skeletal sites, for example, there is less trabecular bone in the shaft of humerus and more in the vertebral bodies(17;51).

2.1.2.2. Bone remodelling

Bone remodelling is a process, by which bone is first absorbed by osteoclasts and then rebuild by osteoblasts. In this way, bone is reformed when necessary to respond to the physical demands placed on it and to repair any microdamage that occurs. The skeleton is made up of millions of basic multicellular units (BMUs) or bone remodelling units, which are usually in a resting stage unless being stimulated by a stimulator (mechanical stress, PTH, local growth factors, etc.). In response to a variety of stimuli these units can be activated and the remodelling process be commenced. There are four distinct steps in the bone remodelling cycle that primarily involve two cell types: osteoblasts and osteoclasts. First step is activation of preosteoclasts and differentiate them to mature osteoclasts. Second step is resorption of old bone by resolving, digesting and absorbing the organic materials and minerals of old bone. Third step is reversal stage, in which once the resorbing cavity reaches a predetermined depth, a cement surface covers the defect and prevents further bone erosion. The final step is formation, in which osteoblasts are attracted into the resorbing cavity and, under the influence of various hormones and growth factors mature and refill the resorbing cavity with "new" bone. These steps take place over a period of approximately four months in a healthy man (497). This cyclic process is repeated until death. It takes place throughout the skeleton and is dominant in trabecular bone, which has ten times the surface area of compact bone. In young adults, this cyclic process is balanced without a substantial net change in the bone mass except for the changes due to growth. In particular circumstances, however, when bone resorption exceeds bone reforming, overall bone loss is expected, such as menopause and inactivity in advancing age. It

has been estimated that at any given time in an adult 3.5% of the total skeleton is being remodelled (36). Mechanical stresses and pressure, hormonal factors and nutritional variables are major determinants of bone maintenance. Any immobilisation such as seen in paraplegic patients or lack of pressure on the skeleton such as weightlessness seen in astronauts can cause bone loss. The effect of pressure is thought to be mediated by piezoelectric signals, by which tells the bone cells when, how, and in what orientation to function in order to adjust the mechanical properties of bone to the need. Bone remodelling is a relatively slow process and the time required to complete a cycle maybe even slower with age.

2.1.2.3. Biologic factors affecting the skeleton

There are several circulating hormones affecting the skeleton, either directly or indirectly via the effect on mineral homeostasis. The secretion of some of them is altered in response to changes in plasma level of calcium or phosphate, (e.g. PTH and calcitonin) and for some others plasma calcium and phosphate are not determinative (e.g. growth hormone, thyroid hormone and gonadal steroids).

2.1.2.3.1. Parathyroid hormone

PTH is a single chain polypeptide hormone, which is involved in the calcium homeostasis in conjunction with vitamin D. It originates from four parathyroid glands embedded in the superior and inferior poles of the thyroid. PTH has a very short half-life as it will be cleared from the blood stream within 8-30 minutes. However, its fragmentation into a number of smaller active fragments causes longer time of action because of longer half-life of these fragments.

The principal regulator of the minute-to-minute secretion of PTH is extra-cellular calcium; dropping the plasma calcium stimulates PTH secretion and vice versa. The key to this regulation is cell membrane Ca^{+2} receptor. When the plasma Ca^{+2} level is high, PTH secretion is inhibited and calcium is deposited to the bones. When it is low, secretion is increased and calcium is mobilized from the bones. Vitamin D directly reduces PTH secretion, whereas increasing the concentration of extra-cellular phosphate stimulate PTH by lowering plasma Ca^{+2} and inhibiting the formation of $1,25(OH)_2$VitD. (52). During hypocalcemia, PTH rapidly releases calcium from bone through its effect on osteocyts

and lining cells on bone surface. Then during a long time (at least 24 hours) it may affect osteoclasts and increase osteoclastic bone resorption. Its fast reaction may occur in the early hours of the morning or possibly after a low calcium meal.

PTH maintains the serum concentration of calcium by actions on bone and kidney. PTH has no direct effect on the absorption of calcium in the gut. Intestinal absorption of calcium is controlled by vitamin D, so the effect of PTH can only be described as indirect, through its control of hydroxylation of 25(OH) VitD in the kidney.

On the kidney, PTH has several actions. It controls the excretion of both phosphate and calcium in the urine. The second effect of PTH on the kidney is increasing the activity of renal1-α-hydroxylase and conversion of 25(OH)VitD to 1,25(OH) $_2$VitD. In the absence of PTH production of 1,25(OH) $_2$VitD will be reduced to negligible levels.

PTH has both anabolic and catabolic effects on bone but with different blood levels. Continuous low levels of PTH can increase osteoblastic activity and may move calcium into the bone, but net removal of calcium from bone occurs in response to relatively high levels of PTH (17;51).

2.1.2.3.2. Calcitonin hormone

Calcitonin hormone is a calcium-lowering hormone, which originates from para-follicular cells of thyroid follicles known as clear cells or C cells. It has a very short half-life (about10 minutes). Its main biologic effect is to inhibit osteoclastic bone resorption. Secretion of calcitonin increases when blood level of calcium rises to about 9.5mg/dl. Many other hormones and factors may increase its secretion such as gastrin, glucagon and oestrogen. Normal calcitonin levels show a circadian variation with a peak around midday. It is metabolised by the kidney and the liver.

Calcitonin decreases the plasma level of calcium and phosphate by direct action on the skeleton and increasing the excretion of Na^+, Ca^{2+} and phosphate through the kidney. However, it has little long-term effect on the plasma calcium level in adults. Within bone tissue, calcitonin decreases the number and the activity of osteoclasts and thereby leads to calcium retention in the bone. It is more active in children than in adults and may play a role in skeletal development.

The plasma level of calcitonin does not respond to oral calcium or food. Postprandial increased blood level of calcitonin is related to gastrin, a hormone secreted from antral portion of the stomach and is the most potent stimulant of calcitonin. This shows an important function of calcitonin, namely, temporarily storing the absorbed calcium during a meal in bone-fluid, for use during the fasting periods between meals (51).

2.1.2.3.3. Effects of other hormones on bone

Various hormones and hormonal factors in addition to PTH and calcitonin affect calcium metabolism and bone maintenance such as cortisol, prostaglandins, thyroid hormones, IGF, growth hormone and sex hormones. Their functions are not primarily concerned with skeletal homeostasis.

Cortisol lowers the plasma level of calcium and inhibits the function of osteoblasts, cellular replication and protein synthesis in bone. It also decreases the absorption of calcium and phosphate from the gut and increases the renal excretion of calcium and phosphate by a partial inhibition of the function of vitamin D. Bone resorption increases under indirect effect of glucocorticoids via hypocalcaemia and secondary stimulation of PTH secretion. Long-term effect on bone formation and bone resorption can lead to osteoporosis. However, normal secretion of glucocorticoids is essential for survival and it does not appear to be a major factor in normal growth.

Growth hormone increases calcium excretion in the urine but it also increases intestinal absorption of calcium and this effect is greater than the former, thus it induces a positive calcium balance. Furthermore, somatomedins (e.g. IGF-I) generated by the action of GH, stimulate protein synthesis in bone and are essential for normal growth.

Thyroid hormones are essential for all cell growth and maintenance but the precise mechanism of this effect is not clear. The excess of thyroid hormones can cause hypercalcemia and hypercalcuria and in some instances, osteoporosis, but the mechanisms remained unclear.

Sex hormones influence the metabolism of vitamin D (see the next section). They also have some effects on osteoblasts and chondroblasts, the cells responsible for growth in length and width of bones. The main effect of oestrogen on bone is on reducing bone re-

sorption by inhibiting osteoclast function and proliferation. Oestrogen deficiency is recognised as the most important cause of type I (post menopausal) osteoporosis. In postmenopausal women, there is an increase in bone remodelling activity, in which bone resorption exceeds bone formation, resulting in a net loss of bone. Androgens in men have similar influence on bone as women. Androgens decrease bone resorption through inhibition of osteoclasts. They might also exert some effects on osteoblasts.

Insulin-like growth factors (IGF I and IGF II) stimulate replication of the osteoblast progenitors and their differentiation and thus, increases the number of osteoblasts (54). They also enhance type I collagen synthesis and inhibit bone collagen degradation. These effects are determinative for the maintenance of bone mass (52). IGF-I is secreted by the liver and its synthesis is growth hormone-dependent. It is also synthesised by various tissues, where may act locally and is regulated by diverse hormones (52). It is proposed that IGF-I mediates the anabolic effects of important skeletal hormones such as parathyroid hormone (PTH), androgens, and oestrogen. Vitamin D can stimulate IGF-I production (55). Meanwhile, IGF-I is one of the main regulators of the renal metabolism of vitamin D (56). It seems that IGF-I is a mediator for the effect of growth hormone on the renal synthesis of 1,25(OH) 2VitD (57).

Other than above mentioned systemic factors, some biologic factors are acting locally to regulate bone maintenance, including interleukins, tumor necrosis factor (TNF), prostaglandins, and transforming growth factor-β (TGF-β) (52).

2.1.2.3.4. Vitamin D

Vitamin D is frequently considered as a hormone, because it can be synthesized in the body and acts as a steroid hormone. It is a fat-soluble vitamin with a variety of functions and two major sources: dietary sources and skin synthesis.

There are at least 37 metabolites of vitamin D of which only three, i.e., 25(OH)VitD, $1,25(OH)_2$Vit D and $24,25(OH)_2$VitD are of particular importance because of their biological activities. 25(OH)VitD is the most abundant circulating form of vitamin D in the blood and is used as a marker of vitamin D status, with a half-life of 2-3 weeks.

$1,25(OH)_2$VitD is the most biologically active form of vitamin D and its circulating concentration is tightly controlled. Normally, the blood level of $1,25(OH)_2$Vit D is extremely low (32±1to 29± 2 pg/ml) with a half life of 14 hours.

$24,25(OH)_2$VitD is produced in the kidney by the 24-hydroxylase or might be a metabolite of vitamin D due to hydroxylation of 25(OH)VitD in target tissues such as the bone, intestine and kidney. Its production is more predominant when vitamin D status is adequate or renal cell cytoplasmic concentration of phosphate is high. Its possible role in bone metabolism has not yet been identified.

Dietary sources

There are two types of dietary sources for vitamin D: animal products (cholecalciferol or vitamin D_3) and vegetable products (ergocalciferol or vitamin D_2). The most common dietary sources of vitamin D across the world are milk and milk products, some cereals and bread products, which are fortified by vitamin D and calcium in some countries such as America. Fatty fish, such as salmon and mackerel, fish oils, meat and eggs are other sources of vitamin D (5). In the U.K. other sources of vitamin D are eggs and fortified foods such as margarine, breakfast cereals and some yogurts(4;58). 97% of older people (>65 years) in the UK had intakes lower than RNI (59). The mean dietary intake of vitamin D in the UK among the elderly population is 2.4μg (SD=1.64) (60), which is less than RNI (10 μg/d). Skin synthesis of vitamin D can not be also a reliable source in the UK because of the northerly latitude (between 50°N and 60° N).

Absorption

Since vitamin D is a fat-soluble molecule its intestinal absorption depends upon fat absorption, which in turn depends on fat digestion mediated by bile salts and the enzyme lipase in the small intestine as well as adequate absorptive capacity of the intestinal wall. In a normal subject about 40% to 90% of ingested vitamin D is absorbed if it is taken in the normal range (5).

Skin synthesis

During exposure to sunlight, the solar ultraviolet B (with a wavelength of 290 to 310 nm) penetrates the skin and leads to photolysis of 7-dehydrocholestrol to cholecalciferol (vi-

tamin D_3). Over a period of 1-2 days this intermediate compound is hydroxylated by the liver and then by the kidneys to create the active form of vitamin D ($1,25(OH)_2$Vit D). In young, light-skinned people about 10 minutes sun-exposure per day on the face and hands may provide enough amounts of vitamin D, but dark-skinned people and elderly people may need more sun-exposure (5). Vitamin D synthesis in the skin may decrease owing to skin pigmentation, skin thickness reduction, using sun-screens and wearing concealing clothes (4;61). However, upper physiologic limit of vitamin D production in the skin is 250μg/day, when the body is totally exposed to the sunlight (62). The amount of produced vitamin D is counterbalanced by vitamin D precursors and some other products such as lumisterol, which are protective compounds against vitamin D toxicity (5;62). Concentrations of these compounds in the skin reach equilibrium in white skins within 20 minute of ultraviolet exposure (62).

This source of vitamin D is a major determinant of vitamin D in the general population (4;63). Because of the northerly latitude of Britain (between 50°N and 60° N) ultraviolet light is at sufficient intensity only between beginning April and mid October (4). Thus, in each year it is mostly inadequate to produce enough vitamin D, and, therefore, vitamin D requirements during winter must be provided from dietary sources or the vitamin store accumulated during the previous summer's exposure.

Storage

Vitamin D as a fat-soluble vitamin is not readily excreted from the body. It is usually stored in the fatty tissues, important storage for maintaining the serum level of vitamin D in various seasons and different situations. Saturation of the fat-tissues prevents vitamin D toxicity during periods of high supply and slow release of vitamin D from this storage provides enough amounts of vitamin D for maintaining its plasma level during periods of low supply. This, provides a physiological explanation for differences in seasonal variations between lean and fat individuals and also shows the importance of skin synthesis in the summer for maintaining the plasma level of vitamin in the winter, when sun exposure is more limited (5;61).

Metabolism of vitamin D

Either coming from diet or the skin, vitamin D is biologically inert and to become active, it must undergo two successive hydroxylations in the liver and the kidney (Figure 2.2). Once entered the circulation, vitamin D (from skin synthesis or taken in with food) is transported to the liver by a binding protein, where is metabolized to 25(OH)VitD by hepatic vitamin D-25 hydroxylase. Hepatic hydroxylase needs NADPH, molecular oxygen and magnesium for its action. This hepatic hydroxylation is not tightly controlled and thus, increase in the intake or the skin production of vitamin D will result in an increase in plasma 25(OH)VitD. This intermediate metabolite is, therefore, used as a marker of recent vitamin D status and composes the major circulating form of vitamin D, although it is biologically inert. Once it formed, it must undergo another hydroxylation in the kidney to convert to $1,25(OH)_2$Vit D by the action of 1-α-hydroxylase, presents in renal mitochondria. This renal function is controlled by the integrated action of PTH, plasma phosphate levels and $1,25(OH)_2$VitD itself. Hypocalcaemia increases the plasma level of PTH, which in turn stimulates renal 1-α-hydroxylase. In contrast, increasing the plasma level of $1,25(OH)_2$VitD directly reduces PTH production within the parathyroid gland and, therefore, indirectly reduces renal $1,25(OH)_2$VitD production. $1,25(OH)_2$VitD also strongly regulates its own renal synthesis directly by its negative feedback regulation on the renal1-α-hydroxylase. Hypophosphataemia can also increase the activity of renal 1-α-hydroxylase even in the absence of PTH. In fact, the cellular level of inorganic phosphate in the renal cells is determinative for 1-α-hydroxylase activity in a negative manner, a procedure, which is mediated by IGF-I (64). It appears that the effect of calcitonin on the metabolism of vitamin D is also mediated by the amount of inorganic phosphate within the renal cells. As stated earlier, IGF-I is a major regulator of renal 1-α-hydroxylase and it mediates the effects of many other regulators such as PTH and growth hormone (GH). Likewise, $1,25(OH)_2$VitD regulates the IGF-I production in the body (Figure 2.2). Estradiol and testosterone can also increase the plasma level of $1,25(OH)_2$VitD via increasing 1-α-hydroxylase and suppression of 24-hydroxylase in the kidney (51). The regulatory function of sex hormones is also mediated by IGF-I (Figure 2.2).

Vitamin D-binding protein

In the blood, vitamin D is mainly bound to its binding protein, with only a small proportion being free and metabolically active. Vitamin D binding protein (DBP) is generally considered as a genetic marker protein, with a single binding site for vitamin D and its metabolites. In normal circumstances only less than one percent to 3 percent of DBP is saturated with vitamin D and its metabolites (65), and hence, even in hepatitis and cirrhosis when DBP drops to 60% of the normal level, the transport of vitamin D is not impeded. It seems that the main site of its synthesis is the liver (66). Animal experimental and human studies indicated that the plasma level of DBP may rise during pregnancy (67;68) and it may drop during undernutrition (69). In fact, fluctuations in the plasma levels of DBP in the absence of hepatic diseases is a physiologic response to the conditions of low vitamin D state or increased need to vitamin D and vice versa (70). Increasing levels of DBP in pregnancy appears to be due to the effect of sex hormone increments in the pregnancy as seen in subjects who are taking oestro-progestogens (65). Serum concentrations of DBP in adults do not follow the seasonal variations seen in the measures of plasma vitamin D (70) and are not correlated with the 25(OH)VitD (65). However, the function of this protein is still controversial, although there is evidence indicating its effect on osteoclast differentiation (71). Genetic polymorphism of DBP is shown to be related to bone mass variance and fracture risk in men (72). This genetical interference in osteoporosis and bone mass variance is also partly through the effect on VDR (vitamin D receptor), which is subject to modification effects of early nutrition in utero and infancy (73).

Reference measurement for vitamin D

Plasma concentration of $1,25(OH)_2$VitD can not be used as an indicator of vitamin D status because it is tightly under homeostatic control. Rather, an intermediate metabolite of vitamin D, namely, 25(OH)VitD, which is not tightly controlled is used as a marker of vitamin D status and it has been shown that the plasma level under 20 nmol/l of this metabolite is associated with clinical presentation of vitamin D deficiency. The level of 25nmol/l of 25(OH) Vitamin D is considered as cut-off point for definition of low vitamin D status by COMA (4) although others suggested higher levels (e.g. 37.5nmol/L by Jacques et al(74)).

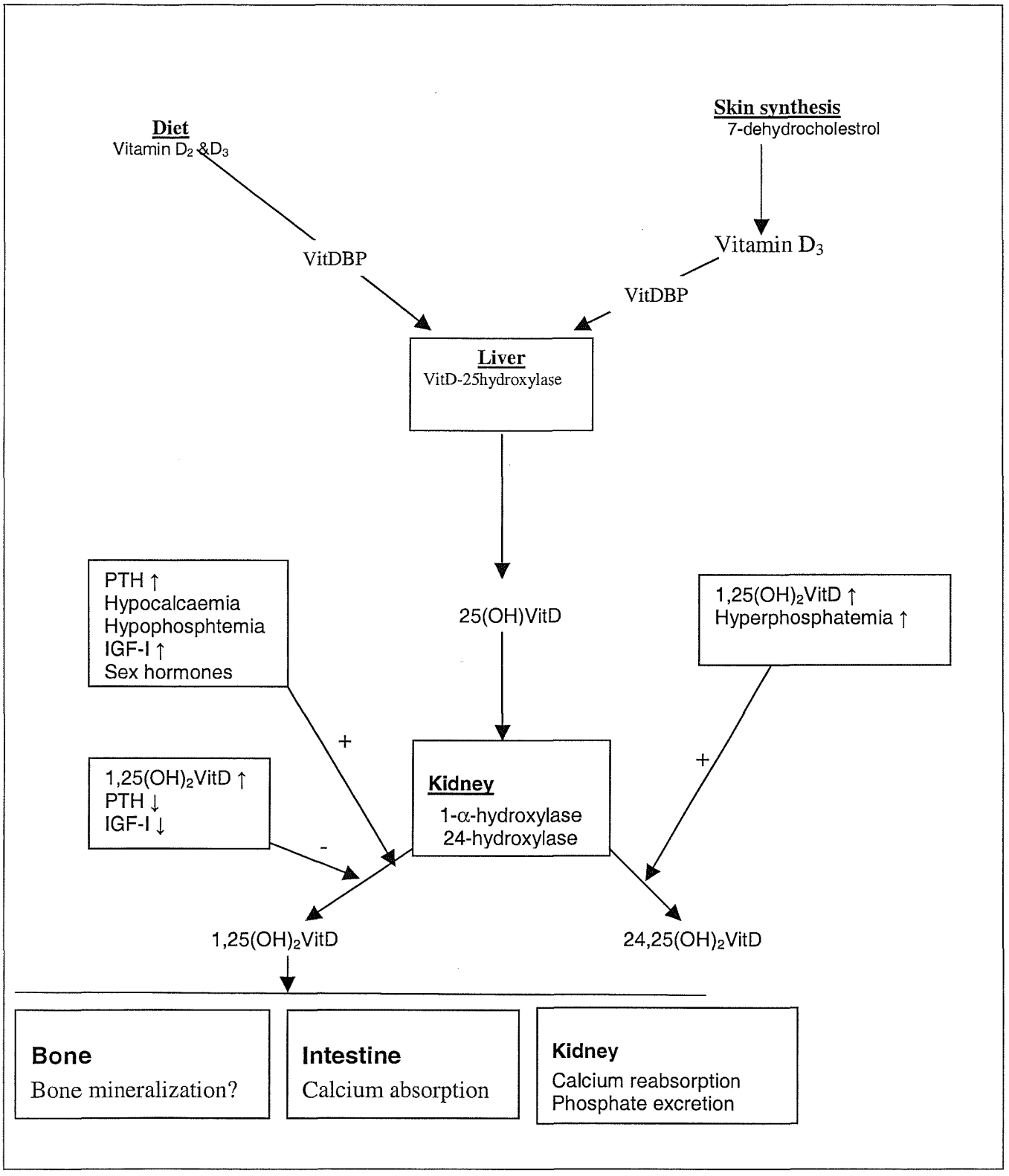


Figure 2. 2- Metabolism of vitamin D.

It has been shown that the plasma level of PTH is also high in vitamin D deficiency situations and may be a marker of vitamin D status, but because this marker is affected by serum concentration of calcium and some other factors and also because of uncertainty in defining a cut-off point for PTH in relation to vitamin D status, it is rejected by Subgroup on Bone Health (working group of COMA) as a marker of vitamin D status in the UK population (4).

Functions of vitamin D

The active form of vitamin D works in conjunction with PTH to maintain calcium homeostasis and bone mineralization by increasing intestinal calcium absorption and reducing renal calcium excretion. They also stimulate the mobilization of calcium stores to the blood via an effect on osteoclasts, phagocytic cells responsible for removing bone tissue, during hypocalcaemia (17).

The mechanism of action of vitamin D is similar to that of steroids. It enters its target cells and interacts with its nuclear receptors by which activates the transcription of specific genes whose products are involved in the biologic responses to vitamin D (52). Interaction between vitamin D and vitamin D receptors (VDR) leads to synthesis of osteocalcin, osteopontin, and alkaline phosphatase within the osteoblast, and calcium binding protein (CaBP) within the intestinal cells (52). The function of these receptors (VDR) is genetically regulated with some modification by environmental factors during early life and neonatal period (73;75-77). It has been suggested that variations in the VDR genes is associated with bone mineral density and the risk of osteoporosis (75), although others did not find such association (78).

Vitamin D is a major determinant for effective intestinal absorption of calcium and phosphate (79). Vitamin D via VDR, increases the production and the activity of several proteins in the small intestine, including alkaline phosphatase, calcium binding protein (CaBP), calmodulin, brush border actin, and some other intracellular proteins, which are important to increase the flux of calcium across the intestinal wall (52). Vitamin D also promotes cell maturation within the intestinal brush border. This is important because intestinal mucosa cells have a short half-life (17).

Vitamin D and PTH are working interdependently in reabsorption of calcium in the distal convoluted tubules and excretion of phosphate in the urine (17). Its negative regulatory feedback in the kidney for the production of $1,25(OH)_2$VitD has already been discussed (Figure 2.2).

Biological effects on bone

Vitamin D and PTH are both involved in bone resorption and their influences depend upon each other. The effects of $1,25(OH)_2$VitD require the presence of PTH, and the effect of PTH on calcium mobilization is diminished in state of vitamin D deficiency. PTH increases the number of osteoclasts and vitamin D increases the size of their ruffled border and their activity. These lead to increase the plasma calcium when dietary calcium is inadequate (17).

The joint action of vitamin D and PTH is also important for bone deposition, directly or indirectly (79). Effects on differentiation of precursors of osteoblasts may provide an explanation (79). $1,25(OH)_2$VitD can also stimulate the production of osteocalcin and osteopontin, which are important for bone mineralization, although the precise mechanism is not well known (80). It has been suggested that osteopontin may be also important for the resorption of bone matrix (80).

Vitamin D is an indirect regulator of calcium deposition in bones especially in the growing ends (17;81). This function may be due to the increase of plasma IGF-I (55). It has also many other functions unrelated to the calcium, such as cell differentiation, immune system regulation, insulin secretion and the effects on nervous system and muscle function (5;52). Effects of vitamin D on muscle mass and the risk of fall is discussed under relating headings.

2.1.2.3.5. Other Vitamins and bone metabolism

Different vitamins play various important roles in bone maintenance:

Vitamin A has been shown to influence both osteoblasts and osteoclasts. Animal experimental studies have shown that a vitamin A deficient diet can lead to a reversible bone overgrowth causing compression of nerves. *In vitro*, vitamin A is associated with bone resorption by increasing the number and the activity of osteoclasts. In addition, it is im-

portant for metabolism of mucopolysaccharides, an essential component of bone matrix. Both excess and deficiency of vitamin A can lead to disturbance of the metabolism of mucopolysaccharide. *In vivo*, vitamin A stimulates PTH secretion and increases the plasma level of calcium (17). Long- standing hypervitaminosis A has been reported to be associated with hypercalcaemia, bone resorption and periosteal calcification. However, both excess and deficiency of vitamin A is not compatible with a healthy skeleton.

Dairy fat, eggs, liver, fortified margarine, many dark greens, red or yellow fruits and vegetables are the main dietary sources of retinol and carotene in the British population. RNI for vitamin A in the UK elderly population is 700μg retinol equivalent/day for men and 600μg for women (58). Mean daily intake of vitamin A in UK elderly population is 1050μg retinol equivalent/day (60). Only 4-5 % of free-living subjects and 1% of subjects within the institutions had intakes lower than LRNI[1] in the elderly in the UK(59).

Vitamin C is concerned with the syntheses of collagen and of glycosaminoglycans. As already discussed vitamin C is essential for the hydroxylation of proline and lysine, which are necessary for the stability of bone collagen. In scurvy, a disease due to lack of vitamin C, there is impaired formation of bone and the failure of bone repair after injury. In this disease, osteoblasts are few but osteoclasts are less affected and complete failure of osteoid formation may occur. The cortex of long bones shows thinning and increased fragility. Probably the most abnormalities seen in scurvy are related to impaired formation of matrix, particularly the impaired synthesis of collagen. However, this is an extreme vitamin C deficiency, which is unlikely to be seen in those with usual dietary intakes.

Fresh vegetables and fruits are the main sources of vitamin C in usual diets. The RNI for vitamin C for elderly men and women is 40 mg/day. The median of dietary intakes from all sources is 38 and 37mg among elderly men and women respectively, in the United Kingdom (60). 2-4% of aged people had intakes lower than LRNI with a negative trend for age (59).

[1] LRNI=The Lower reference nutrient intake. An amount of the nutrient that is enough only for a few people in the group who have low needs.

Vitamin K is necessary for gamma-carboxylation of osteocalcin, an abundant protein of bone matrix (5;58;82). This compound has a high affinity to the calcium in the hydroxy apatite molecule. Additionally, vitamin K may influence calcium absorption in the gut, calcium excretion in the kidney (82) and bone formation in conjunction with vitamin D (5). The classic role of vitamin K is maintain the normal haemostasis by mediation of the synthesis of several blood coagulation factors in the liver.

Vitamin K comes from two sources: dietary intakes and an important proportion, which is made by bacteria in the human intestine. The most nutrient-dense food sources of vitamin K are green leafy vegetables, peas and liver. Between 40 to 80% of dietary vitamin K is absorbed when is taken in usual amounts (5;58). Although it is a fat-soluble vitamin, it could be easily excreted from the body. Most consumed vitamin K during a day eliminates from the body next day (58). Because of abundant dietary sources of vitamin K, clinical deficiency of vitamin K is not common in the UK. In fact, low vitamin K status usually is due to poor general nutritional state (60).

2.1.2.4. Metabolism of bone mineral elements

Calcium, magnesium and phosphate are the main part of bone minerals; fluoride is also a normal constituent of apatite crystal.

2.1.2.4.1. Calcium

Calcium is the most abundant mineral found within the body. In a normal adult, the body contains nearly 1200 gram of calcium, from which, about 99% is stored in the skeleton. The remaining 1 % is circulating in the blood and plays various important roles. Sufficient calcium intake is essential for normal skeletal growth and obtain the optimal bone mass (5;17).

Dietary sources

The major dietary sources of calcium in British population's diet are milk, milk products (approx. 56%) and cereals (approx. 25%, with about 14% from bread due to calcium fortification of "white flour"). Currently, the average calcium intake in the British population is around 820mg/day and for all age groups average calcium intake in women is less than men (4).

Absorption

Calcium absorption is closely related to the gastrointestinal acidity and vitamin D status. It requires a pH below 6 to stay in solution in an ionic state and, thus, the upper part of the small intestine is the best part of GI for calcium absorption, because the intestinal contents become more alkaline as they pass along (5). Calcium is absorbed in two ways; an active transport procedure, which is concentrated in the upper small intestine, is mediated by $1,25(OH)_2$VitD and is saturable, and a passive transport way through GI membranes, which is not saturable and not limited to a specific area, and is less precise (83). The calcium absorption rate in a normal adult is nearly 20% to 40% of ingested calcium, which is about 400 to 1000 mg per day in an ordinary diet. The lowest absorption rate is in postmenopausal women, about 20% and the highest rate is in children during the active skeletal growth periods, about 75 %(5). In normal subjects efficiency of calcium absorption is under physiologic control to meet the body's needs for calcium, it means calcium absorption increases when dietary calcium is restricted or body's needs increased and vice versa (a process called "adaptation") (84). A key factor for this adaptive response is vitamin D (85). Diet composition in terms of sodium, protein, oxalate, phytate, dietary fibre and divalent cation contents (Fe^{++}, Zn^{++}) plays a major role in calcium absorption (4;5). The amount of consumed calcium is also important in regulating the efficiency of intestinal calcium absorption. Fractional calcium absorption increases when calcium intake is low and decreases when calcium intake is high (86;87).

Bioavailability

By definition bioavailability means the proportion of ingested nutrient through the diet that is available for functional purposes. Calcium bioavailability may be affected by different factors such as nutrient interactions, drug consumption and certain diseases. Diet composition plays a central role in this regard. Major favouring factors are vitamin D, PTH, acidity nature of upper intestinal tract and dietary vitamin C, lactose and glucose. Factors limiting calcium absorption include dietary contents of polyphenols (tannins in the tea), excess of phosphorous, iron, and zinc in proportion to calcium, phytic, oxalic and fatty acids as well as dietary fibre (5).

Calcium absorption in the elderly

Calcium absorption declines in advancing age and several factors contribute to this situation such as:

1) Plasma levels of vitamin D in elderly subjects is generally lower than in young adults, thus, the adaptive response is less efficient in advanced age in comparison with younger adults(83).
2) The acidity of intestinal lumen is insufficient in the elderly (5).
3) The absorptive capability of intestinal wall is lower in older subjects than in younger subjects.
4) Since calcium absorption associated positively with activity level and old people are less active, they might be less efficient in calcium absorption (83).

Therefore, people in advancing age are more likely to have a negative balance for calcium. Osteoporotic subjects are at higher risk, because of more insufficient intestinal absorption of calcium (83).

Reference measurement

There is no functional test for calcium status; Plasma level of calcium is under close homeostatic control and it cannot be used as an indicator of calcium status. Other markers of bone health (e.g. BMC, BMD, fractures) and biochemical markers of bone turnover are not directly related to the calcium status, because of several confounding factors influencing them. Therefore, in setting the DRVs (Dietary Reference Values) these criteria are not used and the DRVs for calcium are calculated by other approaches including: factorial approach, balance studies and epidemiological methods (83;88). RNI for calcium in adults is 700mg/day in the United Kingdom and an additional amount of 550mg/day is recommended for lactating women, although there is not enough existing data to support this incremental recommendation (4). The median values of calcium intake among elderly population in the UK are 947mg/day for men and 732 for women (89) with a 5-9% of subjects having less than LRNI (59).

2.1.2.4.2. Magnesium

Beside the constitutional role of magnesium in the apatite crystals, it plays essential roles in many cellular functions. The plasma level of magnesium is relatively constant, even though a precise homeostatic mechanism does not exist.

In a usual diet plant foods are the major sources of magnesium (whole grains, squash, broccoli, nuts, spinach and other greens). PTH and vitamin D may increase its absorption, though the mechanism is not clear (17). Absorption efficiency of magnesium is about 30 to 40 %, which can increase to 80% in low magnesium diets. Approximately one third of extracellular magnesium is bound non-specifically to plasma proteins. The remaining 65%, which is diffusible or ionized, appears to be biologically active. The main excretion route of magnesium is from the kidney, which seems to be the only mechanism of its homeostatic control.

Magnesium is mainly an intracellular ion and its effects on the skeleton are probably dependent upon its actions on intracellular enzymes. It is an activator of a large number of enzymes, some of which are important in the skeleton such as alkaline phosphatase and the pyrophosphatase. On the other hand, it acts as an inhibitor for apatite formation within the matrix vesicles. After breaking the vesicle membrane and losing the magnesium, apatite crystallization would occur.

RNI for magnesium is 300mg/day for men and 270 mg/day for women and the median of its daily intakes is 302 and 226 mg/day among elderly men and women, respectively. 21-23% of aged people consumed less than LRNI for Mg in the UK and this proportion increased with increasing the age (59). Probably the commonest cause of its predominant deficiency is malabsorption, other causes are dietary deficiency resulted from PEM (protein-energy malnutrition) or long time TPN (total parenteral nutrition) and diuretic therapy.

2.1.2.4.3. Phosphate

Phosphate is important for bone mineralization and many cellular activities. It is widely available in diet and some foods such as milk, cheese, bakery products and meat are its major dietary sources. In a normal diet about 70-80 % of dietary phosphate, which is

about 0.5-2gm/day, would be absorbed. $1,25(OH)_2$VitD can increase its absorption and this effect is independent of calcium. No binding protein for phosphate has been identified.

Most phosphate in the plasma is in a diffusible form and only about 12 percent is protein bound. Tissues are less sensitive to changes in phosphate than to changes in calcium levels in the plasma and, therefore, the regulation of the plasma level of phosphate is less precise and mainly is related to the function of the kidney to excrete it (5;58). PTH increases the renal excretion of phosphate.1, $25(OH)_2$Vit D, and 25(OH)Vit D are also important but their effect may be secondary to their effects on PTH secretion.

Besides its roles in many intracellular enzymatic processes, phosphate plays an important role in bone mineralization and hydroxy apatite formation via its effect on solubility product ($Ca^{2+} \times PO_4^{3-}$). However, because of high availability in usual diet, dietary phosphate deficiency has not been reported, but low intakes may contribute to bone loss, especially in some elderly women (58).

Phosphate is found widely in foods and thus, phosphate deficiency is not usually due to dietary insufficiency. Prolonged treatment with phosphate binding antacids and an inherited disease, called vitamin D-resistant rickets are the usual causes of hypophosphataemia. Milk, meat, bakery products and cheese are the main sources of phosphate in the UK. RNI for phosphate is 550mg/day for elderly population, while the median of its dietary intakes is 1429 mg/day, which is much more than RNI (89).

2.2. Nutritional variables and bone health

Most studies of nutrition and bone health have focused on calcium, protein and vitamin D, or isolated foods. Literature regarding to the effects of these nutrients will be presented in this section. General malnutrition, which is a major concern in older people, will be discussed first.

2.2.1. Malnutrition

A precise definition of malnutrition is problematic because a variety of conditions such as imbalance of protein, energy and nutrients might be considered as malnutrition and different screening tests might be used for identification. A simple definition of that is "malnutrition is a condition of impaired development or function caused by a long-term deficiency, excess, or imbalance in energy and or nutrients intake" (5). Negative imbalance of nutritional intakes (undernutrition) is mostly considered as a risk factor for osteoporosis and in this thesis malnutrition is referring to undernutrition. Both specific nutrient deficiencies and general protein-energy undernutrition are common in the elderly and several factors are thought to be in operation such as: various disabilities and different physical and mental diseases, drug consumption, loss of taste, smell and appetite, malabsorption and low physical activity (48;90). With advancing age, decrease in physical activity results in a significant decrease in energy demands and low intake of food, but other nutritional requirements do not decrease with age (48), therefore, dietary intake is more likely to be inadequate in the elderly.

Malnutrition may increase the risk of osteoporotic fractures by accelerating the process of bone loss and altering muscle strength, and thereby, increasing the propensity to fall. It may also lead to a reduction of the protective layer of soft tissue-padding that coveres the skeleton (48;91). The risk of medical complications after fracture and the length of hospital stay can also be increased due to under nutrition (90).

Protein-energy under nutrition may decrease the concentration of vitamin D binding protein (DBP) and, therefore, adversely affect the ability of maintaining adequate concentrations of vitamin D in the blood (69). Another underlying mechanism may be a decrease in

the plasma level of IGF-I (91). Both are important for bone maintenance and muscular performance.

Specific deficiencies of different nutrients are discussed under related headings; here in this section negative balance of nutritional protein-energy intakes is considered as malnutrition unless otherwise is stated.

2.1.1.1. Malnutrition evaluation

Malnutrition can be measured in four main ways:

1) Clinical evaluation including a standardised Subjective Global Assessment (SGA) which incorporates patient history, physical examination findings and the clinician's overall judgement of the person's nutritional status.
2) Anthropometric measurements including BMI (body mass index), mid-arm circumference[2] and TSF (triceps skin-fold) thickness for fat mass, and MAMA[3] (mid-arm muscle area), and mid-arm muscle circumference for muscle mass[4]. Changes in these anthropometric indices are also important, especially weight changes, which may indicate low intakes and under nutrition.
3) Laboratory markers including blood proteins (albumin, pre-albumin and circulating transport proteins), some markers such as plasma fructosamine level (92) and circulating vitamin levels to detect specific deficiencies (vitamin C, D and A), cutaneous antigenic tests and lymphocyte count or lymphocyte function.
4) Food intake measurements, which usually are based on questionnaires by which food intakes during a period of time are measured. 24 recall, 24 dietary record, dietary history, and seven days record dietary intake are some examples of such measurements (93).

All above mentioned tools have their own limitations and no single one is superior. Mid-arm muscle circumference is a useful measure of muscle protein stores, unintentional weight loss is a dynamic measure of nutritional status and measurement of triceps skin fold thickness provides an estimate of body fat reserves. However, because of changes in fat distribution and skin thickness due to advancing age, TSF thickness may not be ideal

[2] arm muscle circumference = arm circumference $-\pi$ TSF
[3] arm muscle area = (arm muscle circumference)^2 / 4π
[4] corrected arm muscle area

for assessing fat mass in older people and maybe better to assess fat mass by trunk adiposity measurements such as abdominal skin-fold thickness (93). The main limitations of dietary assessment methods are dietary variation, measurement errors and the errors of respondents to provide precise data (94).

Recently, the malnutrition advisory group (MAG) has developed guidelines for the detection and management of undernutrition in the community, in which a combination of unintentional weight loss and BMI would define the risk of undernutrition (95-97). These guidelines have been used to evaluate the risk of malnutrition and its relation to health in people aged 65 years and over in the UK (98). Significant associations between defined undernutrition risk and plasma markers of different nutrients, energy intake and the health status of the subjects in this study, indicate that these guidelines may be reliable in defining the risk of undernutrition. However, it seems that more extensive studies among different populations are needed to show the validity of this approach for different populations.

2.1.1.2. Prevalence of malnutrition in osteoporotic patients

Depending on selected criteria and population, different rates of malnutrition amongst osteoporotic patients have been reported.

Prevalence of malnutrition in hip fractured elderly patients in the UK has been reported to be high. Using TSF thickness and MAMC in a study on 744 hip fractured elderly women, Bastow et al (99), have reported a prevalence of 53% for undernutrition. They defined undernutrition on the basis of TSF thickness and MAMC as being more than one standard deviation below the mean of the reference population, defined as normal elderly population (in the UK or Belfast). In 18.5% of patients both indices had values more than two SDs below the mean of reference population. The reference population in this study can be treated as a control group, although one part of it (elderly population from Belfast) may be varied from the study population in terms of social, economical and genetical (for body build) background. The prevalence of undernutrition among hip fractured patients in this study is comparable with the prevalence of undernutrition among the elderly population in the UK, reported by Margetts et al (98), who showed that about 14% of elderly population were at medium or high risk of undernutrition defined by MAG tool. Although, their crite-

ria for the risk of malnutrition is different from that of this study (99), the big difference between prevalence of undernutrition in the UK general population and in hip fractured patients indicates higher prevalence of undernutrition in these patients. However, TSF thickness in this study might not be a good indicator for undernutrition and, therefore, the reported prevalence may be overestimated (93). This study has indicated that mortality rate after fracture was much higher in malnourished (very thin) subjects compared to the well-nourished ones (18%v.4.4%).

Similar results were shown in another study conducted in New Zealand. Considering three criteria (pre-albumin, TSF or CAMA) for assessing nutritional status, Hanger et al (50) measured the prevalence of malnutrition in 66 hip fractured patients aged over 65 years (mean age 81.5 y). They defined malnutrition as a low result in at least two of the three indices of nutrition (pre-albumin, TSF or CAMA). Lower limit for pre-albumin was defined as 0.20 g/L and for TSF or CAMA was defined as 5^{th} percentile for age-sex reference data or lower (100). Using these criteria, PEM was present in 42.4% (95%CI, 29.8-55) of patients. In 8.5% of patients all three indices were low. In this study, all indices of malnutrition were negatively correlated with increasing age but there were no statistically significant correlation between the serum proteins and either TSF or CAMA. However, response rate in this study was 77.6% and TSF as a marker of body fat in this population may have not been a precise measure.

Another study by Jamal et al (92) reported a higher risk of hip fracture among malnourished than well nourished subjects, using a plasma nutritional marker (serum fructosamine level). This study was conducted on 477 women aged 65 years and over (100 cases of hip fracture, 101 cases of vertebral fracture and 276 controls) and malnutrition was defined as being in the lowest decile of serum fructosamine level. Undernourished subjects were at a three-fold increased risk of hip fracture (95% CI, 1.4-6.4, P= 0.004), compared with all other women, after controlling for age, weight, and hormone replacement therapy. For vertebral fractures, however, the association between serum fructosamine and the risk of fracture was not statistically significant, although there was a slightly higher risk of vertebral fractures among malnourished subjects in comparison to all other subjects (OR = 1.7, 95%CI, 0.8-3.8). When women with fructosamine levels in the three lowest deciles com-

pared with the others, there was an increased risk for them to have a vertebral fracture (OR = 1.8; 95%CI: 1.0-3.2; $P = 0.05$).

2.1.1.3. Summary

Summing up, malnutrition is common in the elderly; in particular among those who suffer from osteoporotic fractures. All presented studies have confirmed that undernutrition is associated with frailty fractures, since the prevalence of undernutrition is higher in fractured patients(50;99) and also the risk of fracture is higher for malnourished subjects (92) when compared to the well nourished ones and thus, it was referred to as a risk factor for bone health in almost all studies. However, differences in definitions of malnutrition made comparison across the studies difficult. Therefore, a clear and biologically relevant measure for nutritional state in any research on diet and bone health is necessary. In all presented studies, the reference population was general healthy people of the same age ranges and mostly from the same area.

2.2.2. Protein

Protein is an essential component of bone and comprises more than 1/3 of bone mass. As such, bone tissue is one of the most protein-dense tissues of the body (102). Protein is also necessary for maintaining the plasma level of IGF-I, a growth factor that exerts an anabolic effect on bone. IGF-I is essential for bone longitudinal growth, production of $1,25(OH)_2VitD_3$ and renal absorption of phosphate (48;52;54-58). Adequate protein intake also is essential for muscle mass and muscle strength, which provide protective mechanisms to reduce the impact of trauma on the skeleton and also is important for the risk of fall (48;49). On the other hand, high protein intakes may increase urinary calcium excretion via elevating metabolic acid production (103;104) and chronic bone buffering and bone dissolution.

2.2.2.1. Protein and calcium

Calcium-protein interaction in relation to bone metabolism has long been investigated. Both calcium and protein are important structural components of bone and their metabolic interactions may be determinative for bone health. The main features of studies about the effects of protein on urinary calcium excretion and intestinal calcium absorption are presented in Table 2.1.

Table 2. 1- The effects of protein on intestinal calcium absorption and urinary calcium excretion

Author	Subjects			Study design	Outcome measure	Results
	n	Sex	Age(y)			
Hu et al(103)	764	F	35-75	Cross-sectional	Urinary calcium	Calciuric effect of animal protein. r =0.17, $P<0.01$
Kerstetter, et al (113)	7	F	21-39	Experimental	Urinary calcium and FICA[1]	Increased both FICA and calcium excretion by increasing protein intake, with negative calcium balance.
Kerstetter et al (115)	8	F	20-40	Experimental	Calcitropic hormones[2]	A threshold effect of protein on calcitropic hormones, at a level of 0.9g/kg body weight protein intake
Lutz,(116)	8	F	Postmenopausal	Experimental	Urinary calcium, radial BMC and FICA[1]	Increased both FICA and calcium excretion by increasing protein intake, with no effect on radial BMC, vitamin D and PTH.
Heaney (117)	191	F	35-41	Experimental	Calcium absorption	No effect of protein on calcium absorption.
Spencer et al (118)	14	M	40-67	Experimental	Urinary calcium and calcium absorption	No effect of protein on calcium balance.

1. FICA =fractional intestinal calcium absorption. 2. Measured calcitropic hormones were PTH, $1,25(OH)_2$Vit D and nephrogenous cyclic adenosine monophosphate.
F= female, M= male.

Hu et al(103) found that urinary calcium excretion was significantly correlated with total protein intake, after adjustment for age and body weight, ($r = 0.25$, $P < 0.01$)(103). Meat and non-dairy animal foods (NDAF) were positively associated with urinary calcium excretion ($r = 0.17$, $P < 0.01$). Corresponding association for plant foods was negative and significant ($r = -0.16$, $P < 0.001$). Therefore, it was concluded that the observed calciuric effect is due to the animal component of dietary protein rather than plant component. The increase of calcium excretion was independent of dietary calcium intake. In this study, however, other dietary variables were not taken to account, for example; phosphate, and dietary carbohydrates are important components, which may vary among different calcium quartiles and are known to affect intestinal calcium absorption and urinary calcium excretion (112). Vitamin D adequacy as one of the main regulators of calcium metabolism was not controlled for, and the wide age range of subjects included in this study, making it difficult to disentangle confounding by age and sex hormones. Furthermore, although analyses were controlled for dietary calcium, it cannot dismiss increased calcium absorption as a source of excess calcium excretion, since it has been shown that fractional cal-

cium absorption increases when calcium intake is low and it decreases when calcium intake is high (86;87). In other words, from these results it cannot be concluded that excess protein intake causes negative calcium balance, or mobilisation of bone calcium, which is harmful for bone maintenance.

In this respect, a further study by Kerstetter, et al (113) investigated the possible source for calcium, excreted in urine, following a high-protein diet. Comparing of two levels of protein intakes (low: 0.7 g/kg; and high: 2.1 g/kg) showed that after four days of consuming these experimental diets, urinary calcium excretion was significantly higher in high-protein diet than in low-protein diet (difference = 2.2 ± 0.5, $P = 0.005$). Serum calcitropic hormones (vitamin D and PTH) increased significantly at day 4 in subjects consuming low protein diet compared to subjects using high-protein diet. Fractional intestinal calcium absorption was significantly higher with the high than with the low protein diet (0.26±0.03 v. 0.19±0.03). These results indicate that increase in urinary calcium excretion following a high-protein diet is, in part, due to increased intestinal calcium intake. Up regulating of the plasma vitamin D and PTH in those consuming low-protein diet and their less calcium absorption than the high protein diet indicated lower calcium status and probably bone catabolism situation in these subjects. This was confirmed by another study conducted by the same investigators (114).

The effect of protein on intestinal calcium absorption had also been reported by an early study (116), in which eight postmenopausal women received two diets containing two levels of protein (110 and 50g/d), randomly. The calciuretic effect of high protein diet was accompanied by an increase in net calcium absorption from the gut, while calcium, magnesium, and phosphorus intakes were held constant at 713, 323, and 1078 mg/day, respectively (Table 2.1).

In this regard, a recent study suggested a threshold effect for dietary protein intake of about 0.9 to 1g/kg body weight (115) by comparing the effects of four experimental diets containing four levels of protein: 0.7, 0.8, 0.9, 1.0 g/kg weight on calcium metabolism. Increment of calcitropic hormones happened with protein intake of 0.7 and 0.8 g/kg but not with 0.9 and 1.0 g/kg. Hence, they suggested an allowance of 0.9 to 1.0g/kg body weight for optimizing calcium balance, and preventing bone loss.

However, these results are contrasting with some other studies, which have reported no effect of protein intake on the intestinal calcium absorption. Heaney (117) found no difference in calcium absorption between subjects consuming high- or low-protein diets. No relation between calcium relative absorption and either phosphorus or protein intake was detected in both pre and postmenopausal women, analysed separately or altogether. No threshold effect of protein on calcium absorption was also found in this study for both groups of women.

Similar results have been reported by Spencer et al (118). In this study, the effects of three levels of protein intake (2g/kg body weight for high protein, 1g/kg for control studies and 0.5 for low-protein studies) during a low calcium intake (>200mg/day), a normal calcium intake (800mg/day), moderate (1100 mg/day) and high calcium intake (>2000mg/day) on calcium absorption, retention and excretion were examined. High protein intake during low and normal calcium intake periods was not associated with a significant increment in urinary calcium excretion compared to the control studies. During high protein intake and high calcium intake (2140mg/day) although urinary and faecal calcium excretion increased significantly, calcium balance remained unchanged. During the normal calcium intake of 800 mg/day no significant difference was found between high and control protein intake in terms of urinary and faecal calcium excretion and calcium balance. During calcium intake of 1100 mg/day and high protein intake, calcium balance increased slightly. During low protein-low calcium studies the calcium balance became slightly negative. Low protein-intermediate calcium studies found no change in calcium balance though the urinary and faecal calcium excretion increased. Low protein–high calcium studies found a slight positive balance of calcium. Similar studies by the same investigators on 4 healthy men for 78-132 days and also on 3 men for a short period of 18 to 32 days (119) found no effect of high protein intake on calcium excretion, absorption and balance during both short and long periods. In summary, Spencer and collaborate have dismissed the results of previously mentioned studies indicating that protein intake may increase calcium excretion and induce a negative calcium balance.

There are important discrepancies between the results of the presented studies. Differences in methodology and limitations of each study are of importance and could account

for the discrepant results. The first study presented here (103) reported high calcium excretion with high protein diet. Difference between absorbed and excreted calcium (calcium balance) is not determined and most importantly, it is not clear to which extent bone metabolism wa affected by increased protein intake or increased urinary calcium excretion. Wide age range and missing a numerous confounders are other limitations of this study. Kerstetter et al (113) reported studies on healthy young women while, Spencer et al conducted their studies on old male with a variety of medical problems, which were not taken into account, and making it difficult to compare the results. The main limitation of the presented studies is their approach of considering protein in isolation and neglecting other components of the evaluated or applied experimental diets. For example, two experimental diets (low and high protein diets) in the study of Kerstetter et al (113), cited above, were significantly different in carbohydrate content and carbohydrate is known to affect calcium metabolism (112). They were also significantly different in terms of the quality of protein as the main nutrient, concerned with this research. Additionally, Heaney (117) evaluated only dietary protein, calcium and phosphorus. Dietary protein, however, comes in the form of foods that contain associated nutrients, which maybe important for bone health and calcium metabolism. Vitamin D and zinc are some examples of such nutrients, which may vary by protein variations.

Other speculated association between calcium and protein is the modifying effect of calcium on the relations between protein and bone. This was examined by Dawson-Hughes and Harris (120). 342 men and women aged 65 and over completed a 3-y randomised, placebo-controlled trial of calcium and vitamin D supplementation. Treatment group received calcium (500mg/day) and vitamin D (700 IU/day = 17.5 μg/d) supplementation and controls received double placebo. BMD of the femoral neck, spine, and total body were measured every 6 months. Within the supplemented group, changes in BMD over 3 years were positively associated with protein intake, whereas within the placebo group there was a non-significant but negative association between protein intake and changes of BMD. Furthermore, within the supplemented group low protein intake was associated with bone loss, indicating that calcium may not be enough to protect the skeleton when protein intake is low. Age, sex, weight, total energy intake, and dietary calcium intake

were taken to account. Changes in BMD at lumbar spine were not associated with protein intake in either treatment group. These relations were not significant for animal protein, suggesting that the total amount rather than the source of protein may be important. Absorbed calcium did not differ across the thirds of protein intake within the supplemented group, while it decreased with increasing protein intake within the placebo group.

It is noteworthy that the calcium supplement in this study was calcium citrate malate, which included base precursor and, therefore, may have influenced urinary calcium excretion and interfere with the results. Furthermore, BMD changes may not be precisely measurable as BMD itself, and may be subject to information bias due to changes in covariates during the time. Additionally, BMD itself may impact the rate of bone loss, as women with higher BMD have been observed to lose their bone, slightly faster than others with lower initial bone mass (121). Finally, dietary intake was assessed by a FFQ, measuring usual intake for past 12 month, but calcium absorption is related to the diet at the time of absorption measurements, thus associations between protein and calcium absorption may be obscured by daily variation in dietary intakes.

However, others found the opposite. A recent study by Promislow et al (122) has reported effect modification of calcium on the relations between protein and bone health in 960 men and women aged 55-92 years, who participated in the Rancho Bernardo Study. Using dual-energy x-ray absorptiometry, BMD of the hip, femoral neck, and lumbar spine were measured at the baseline and four years later, where total body BMD measurement was also added. Calcium modifying effect was evident only in women and for total, animal and plant proteins; as calcium intake decreased, the association between protein intake and BMD became more positive. This indicated that increased protein intake particularly is beneficial for women with lower calcium intakes. After four years, changes in BMD were not significantly associated with animal, plant and total protein in both sexes. Limitations of this study are discussed in the next section.

There are substantial differences between these two studies that could account for the discrepant results. The study by Promislow et al (122) was performed in subjects with mean calcium intakes of 985 mg/day, whereas the supplemented group in the study of Dawson-Hughes and Harris on average consumed 1346 mg calcium per day and the placebo group

consumed 870mg/day. If there is a threshold effect for calcium, it may explain these discrepancies. Furthermore, Dawson-Hughes and Harris analysed men and women altogether while the effect may not be similar in sexes, as shown in the study of Promislow et al. Both supplements of calcium and vitamin D in the study of Dawson-Hughes and Harris may affect obtained results; citrate malate is an alkali and attenuates protein-induced acidosis and bone buffering. Vitamin D is also determinative for adaptive responses of intestinal calcium absorption and urinary calcium reabsorption (83;85). Finally, fractional calcium absorption from supplement and diet may be different due to different calcium bioavailability (123).

With respect to the protein-calcium interaction Heaney (102) suggested that the effect of dietary protein is related to the ratio of dietary calcium /protein. He has suggested that a ratio of 20:1(mg:g) may be compatible with a good bone health. Feskanich et al (124) and Cooper et al (105) did not find such a relationship, while Metz et al (125) have reported a positive correlation between bone health and calcium/protein ratio.

Beyond protein-calcium interactions, it is important to know the extent to which protein may influence bone health and fracture risk in the community.

2.2.2.2. Protein intake and bone health

Associations between protein and bone health in the elderly has been widely investigated in recent decades. It is well known that protein has an important influence on skeletal health but the nature of this effect has remained controversial. Key findings and main aspects of published studies are presented in Table 2.2.

Geinoz et al (49) reported that lower protein intake, which was defined as intakes of less than 1g/kg of ideal body weight daily, was associated with lower BMD at the femoral neck and lower physical performance (as evaluated by capability to walk and climb stairs without stopping) in both sexes. Conversely, high protein intake (≥1gr/kg/d) was associated with higher BMD at the femoral neck (mean of 0.68 g/cm^2 in the high intake group Vs. 0.57 in the low intake group in women and 0.76 Vs 0.64 in men, [P <0.05]). 8/9 of hip fractured women were found to belong to the low dietary intake group. In this study, however, the study population was selected from hospitalised patients and thus was not

similar to the general population. Furthermore, dietary intake in hospital and after affection may not represent their usual dietary habits.

Table 2. 2-Studies of protein consumption and bone health

Author	Subjects			Study design	Dietary Ass	Outcome measure	Results
	n	Sex	Age(y)				
Geinoz et al (49)	74	F/M	>85	Prospective (28-d)	Recorded diary	BMD of femur and spine	Positive association between protein and BMD
Munger et al (106)	41837	F	55-69	Prospective (3-y)	FFQ	Hip fracture risk	Reducing the risk of hip fracture by increasing protein intake
Promislow et al (122)	960	F/M	55-92	Prospective (4-y)	FFQ	BMD of hip femur and spine	BMD was positively associated with animal protein and was negatively associated with vegetable protein. No association between protein and BMD changes.
Cooper et al (105)	290	F		Cross-sectional	FFQ	BMD of femur, spine and radius	BMD was positively associated with protein intake only in premenopausal women.
Feskanich et al (124)	85,900	F	35-59	Prospective (12-y)	FFQ	Hip and forearm fracture risk.	Increasing the risk of forearm fracture with high protein intake. No effect on RR of hip fracture.

F= female, M= male, FFQ= food frequency questionnaire.

A further study by Munger et al (106) showed a beneficial effect of protein intake (especially, animal protein) on the risk of hip fracture among postmenopausal women. RR of hip fracture in the group of the highest protein intake in comparison with the lowest protein intake group was 0.31 (95%CI, 0.10-0.93 P=0.037). Age, number of pregnancies, BMI, smoking, alcohol use, oestrogen use and physical activity were taken to account. Animal protein consumption was positively correlated with dietary calcium (r = 0.32, P <0.0001) and vitamin D intake (r =0.28, P <0.0001) but negatively with vegetable protein consumption (r =-0.43, P <0.0001). They found no significant effect for vegetable protein on the risk of hip fracture, possibly because of low vegetable protein consumption in their population (73% animal protein vs. 27% vegetable protein). In this study however, the response rate was low (42%), and rather small proportion of hip fractured cases were analysed (44 of 125hip fracture, [35%]), which might introduce bias.

Recently, Promislow et al (122) reported a positive association between animal protein intake and bone mineral density (BMD) of the hip, femoral neck, and lumbar spine, measured over a period of 4 years. Vegetable protein intake was negatively related to

BMD at all measurement sites except that of the total body. Corresponding associations in men did not reach to a statistically significant level. However, changes in BMD over four years were not significantly associated with animal, plant and total protein in both sexes. Response rate in this study was rather low (60-65%), and non-respondents were significantly different from respondents in terms of protein intake, medications and physical activity, which may have introduced selection bias.

In accordance with the above-sited studies, Cooper et al (105) published a study, in which two groups of women (post and pre-menopausal women) were examined regarding to the effect of dietary protein on BMD of lumbar spine, three femoral sites (neck, shaft, trochanter), the distal and the mid-radius. Analysis of these two groups showed a favourable effect of protein intake on bone mass only in premenopausal women (n =72). There was also a positive relationship between protein intake and BMD at five out of the six skeletal sites among postmenopausal women (n=218) but did not remain significant after adjusting for age, body weight, and physical activity. Among *premenopausal* women, protein intake was positively related to the BMD at all six sites: correlation coefficients (r) for lumbar spine, three femoral sites (neck, shaft, trochanter), distal and mid-radius were equal to 0.07, 0.27(P<0.05), 0.16, 0.35 (P <0.01), 0.28(P <0.05), and 0.21 (P <0.05), respectively. These relationships were adjusted for weight, age and physical activity. In *postmenopausal* women the positive relations between dietary protein and BMD did not remain significant after adjusting for weight, age and physical activity. Both pre and postmenopausal women with osteoporotic fractures had lower protein and phosphorus intakes, compared to non-fractured ones. There was no relationship between bone density at any skeletal site and calcium/protein ratio. Separate analysis for post and pre-menopausal women, accounting for a vast range of variables (especially the lifestyle variables) and considering numerous skeletal sites may be considered as the strengths of this study. The low response rate (60%) (Probability of selection bias) on the other hand, and low statistical power of study for premenopausal women (56% to detect correlation coefficient of 0.25 at the 5% significant level) were the main limitations.

Contrasting to these studies, Feskanich et al (124) found a positive association between forearm fracture risk and protein intake, although association with hip fracture was not

significant. In this study, protein intake was associated with increased risk of forearm fracture but no significant association was found between protein intake and the risk of hip fracture. Adjusted relative risk of forearm fracture was 1.18 (95% CI 1.01-1.38), while similar RR for hip fracture was 0.79 (95% CI 0.53-1.19) when women in the highest quartile of protein intake (>95 g/day) were compared with those within the lowest quartile (<68g/day). Separate analysis for animal and vegetable proteins showed that the observed associations were due to animal proteins rather than vegetable proteins. Plant protein had no association to fracture risk. The ratio of dietary calcium intake to protein intake (calcium/protein) was not significantly related to the risk of forearm fractures (RR= 0.91, 95% CI 0.78-1.06 when the highest ratio group (11 or more) was compared with the lowest one (<5)).

In all presented studies, protein intake was positively and significantly correlated with dietary intakes of calcium and vitamin D.

Summing up all these studies generally showed a positive relationship between dietary protein and bone health except one prospective study (124), in which the risk of forearm fracture increased slightly with protein intake. Differences in methodologies and subject characteristics made it difficult to compare the results. However, none of the presented studies showed negative association between BMD and protein intake and none of them showed adverse effect of protein on the risk of osteoporotic fractures.

2.2.2.3. Protein supplementation

Two prospective studies by Bonjour et al (48;126) investigated the relationships between protein supplementation and bone health. These studies have shown favourable effects of protein supplementation on the clinical outcome of hip fracture. In the first study (126), 59 hip fractured patients (27 supplemented and 32 control subjects) were recruited. The former group received a dietary supplement including different nutrients (e.g. lipid, protein, various vitamins and minerals), by which the energy intake increased by about 25%, protein by 60% and calcium by 130%, during about 30 days supplementation. The rate of complications (e.g. bedsore, severe anaemia, lung and renal infections and so on) and mortality were lower in the supplemented subjects than in controls (44% in supplemented group vs.87% in controls). After 6 months of discharge from hospital there was no death

in supplemented group in compare with 6 in controls (19%). This study also showed a long-run beneficial effect of supplementation with regard to the complication rate after 6moths (40%vs.74%).

In the second study by these authors (127), in order to identify the key nutrient responsible for the mentioned favourable effect of the supplement, they conducted another randomised-controlled study on 62 patients with hip fracture (mean age 82), who were randomised into two groups: treatment group (n = 33) who received dietary supplement containing protein and control group (n =29) who received the same dietary supplement, but without protein. The rate of complications and death was significantly lower in the protein-supplemented group compared with the control group (52% Vs 80%, P <0.05) 7months after hip fracture. The median hospital stay was significantly lower in the protein-supplemented group (69 vs. 102 days P<0.05). Therefore, they concluded that the favourable effect of their supplement was mainly related to the protein component, rather than other nutrients. However, they stressed that their oral supplement increased the overall protein intake only up to the level of RDA (in USA) for protein (0.8 gm/kg body weight). From this study, however, it is not clear how many cases were malnourished and whether these supplements could be applied to all patients with hip fractures or just malnourished patients will benefit from them.

2.2.2.4. Serum protein levels and bone health

Serum protein concentration is an indicator for protein nutritional state (93;128). The relationship between bone health and serum protein concentrations has been investigated in two studies. In the first study, Orwoll et al (128) examined the association between bone mass and serum albumin levels in two groups of normal *men,* separately. The two groups were included 62 and 92 normal men (subjects without apparent disease, or evidence of osteopenia) aged 30-90y. Serum albumin levels were positively correlated with the BMD of proximal and distal radial sites in the second group (r = 0.22 and 0.28 P<0.05 and P<0.01, respectively) and with vertebral BMD in both groups (r = 0.60 and 0.46, P<0.01). They also showed a positive correlation between dietary protein intake and serum albumin level as well as positive correlation between protein and calcium intakes. Age was negatively associated with serum albumin (r = -0.65, P<0.01) and BMD at all skeletal sites.

Dietary protein was also related to vertebral and proximal radial sites but not with the distal radial site. In this study however, only protein, vitamin D and calcium as dietary variables were considered. Furthermore, albumin is a negative acute phase reactant, therefore, is affected by factors other than nutritional variables, such as infectious diseases and inflammations and, therefore may not be a reliable marker of nutritional state. These possibilities were not taken to consideration in this study, although it was emphasized that the subjects were free of diseases.

Long et al (129) have reported a case-control study, in which 10 hip fractured women (aged 70-92y), one day after surgical fixation during both a 24-h fasting state (day 1) and while receiving total parenteral peripheral nutrition (TPPN) (day 2) were compared with 19 healthy women as controls (aged 70-84y), who experienced similar fasting and TPPN periods. TPPN solution provided 1.5g/kg amino acid and 29-30 kcal/kg daily. Authors observed lower total plasma amino acid concentrations and plasma albumin levels in the hip fractured patients in comparison with healthy control subjects. After 24-h fasting plasma albumin concentration was 28±1and 40±1(p<0.001) in injured and control subjects, respectively, and the total amino acid (TAA) pool of the trauma group was significantly lower by 17% compared with the control group. After TPPN (on day2) albumin concentration was still low in injured subjects (24±2 vs.40±1, p<0.001) and TAA pool was still lower than controls (by 20%). The small number of subjects in this study has limited the generalizability of the findings. Furthermore, cases and controls might be different with respect to other health aspects as the cases were suffering from a fracture, which is an apparent stress for them and may affect plasma levels of proteins as well as dietary intakes.

These two studies showed the positive correlation between bone health and protein nutritional state in men and women; although a few variables were considered.

2.2.2.5. Teenage protein intake and bone health in the elderly

Teenage protein intake has been shown to have no effect on bone health in the elderly. Feskanich et al (124) in their study, stated above, showed no statistically significant association between teenage protein consumption and fracture risk in the elderly. The relative risk of forearm fracture for women who consumed more than 70 g/day in their teenage

period compared with women who consumed 30 g or less was RR = 1.03, (95% CI 0.86-1.25, P = 0.58). Hip fracture RR = 0.64 (95% CI, 0.38-1.10, P = 0.28).

In this regard, Metz et al (125) conducted a cross-sectional study in 38 Caucasian women (aged 24-28y) to explore association between protein intake and bone density in the third decade. They observed a negative association between protein intake and radial BMD during the third decade in women (P<0.05) and concluded that the protein intake higher than recommended amounts may adversely affect radial bone density. The ratio of calcium/protein intake was positively related to the distal and mid radial BMC (P=0.01). High calcium intake (30% above the mean intake of USA women and for UK population) in this study limited the generalizability of the findings.

The results of these two studies showed no beneficial effect of teenage protein intake on bone at femoral neck and a possible negative effect of high protein intakes (more than 0.80 gr/kg BW) on the radial bones. The effect of protein intake in early life on osteoporosis in the elderly is remained to be discovered.

2.2.2.6. The importance of dietary source

An important issue about dietary protein is the source of protein. Abelow (130) from a cross-cultural analysis suggested a significant positive association between animal protein and hip fracture risk in women over 50 years old. However, inconsistent information has been published in this regard. Munger et al (106) in the study stated before, have reported that animal protein intake was negatively related to the hip fracture risk. Feskanich et al (124) have reported no increase of hip fracture risk by animal protein consumption (RR=0.84 95% CI 0.49-1.44) whereas the risk of forearm fracture slightly increased with animal protein intake (RR= 1.21, 95% CI 1.03-1.41 P = 0.01) and in both studies (106;124) no relationship between vegetable protein intake and hip or forearm fracture risk was observed.

Tesar and colleagues (131) have reported no difference between vegetarian and omnivorous women with respect to their bone health. In his study 28 lacto-ovovegetarian women (who practised this dietary pattern for more than 10 years) were compared with a group of omnivorous women, who were matched by age, weight, height and years since meno-

pause. All subjects were aged between 55 to 75 years and at least one year after menstrual cessation. BMD and BMC were measured at forearm, lumbar spine and entire skeleton. No significant differences in bone measurements were shown between vegetarians and omnivorous women despite differences in dietary intakes. Differences in dietary intakes and possible differences in lifestyle variables may have conflicted the results.

Sellmeyer (132) published a prospective study in 1035 women aged >65 y for a period of about 7 years. After adjustment for a number of potential risk factors, there was no association between BMD at hip and the ratio of animal to vegetable protein. In contrast, femoral bone loss was significantly related to the ratio of animal to vegetable protein. Women with a high ratio of animal to vegetable protein intake had a significantly higher rate of femoral neck bone loss than did women with a low ratio (0.78%/y and 0.21%/y, respectively). Analyses of 48 hip fractured women showed that the risk of hip fracture was significantly higher in women with a high intake of animal protein than in those with a low intake (RR = 2.7, $P = 0.04$). In contrast, women with a high intake vegetable protein had an RR of hip fracture of 0.30 ($P = 0.03$). Women in the highest quintile of ratio of animal to vegetable protein intake (3.17) had nearly a 4-fold greater risk of hip fracture compared with women with low ratios.

Dawson-Hughes and Harris (120) in their study, stated above (see section 2.2.2.1), found no difference between plant and animal protein regarding to the BMD changes of a number of skeletal sites over a 3-y period in subjects supplemented with vitamin D and calcium.

In interpreting these studies, it is important to differentiate between BMD, BMD changes and the risk of fracture. BMD is a marker of bone health indicating the bone strength, but measuring the changes of BMD is less accurate than measuring BMD itself, (133) and is subject to information bias due to changes in covariates during the time of the measurements. Risk of fracture on the other hand, is the function of both bone strength and the risk of fall, which is related to the muscle performance. Muscle performance is in turn, influenced by many factors other than nutritional ones. The first three studies presented here (106;124;130) have reported relations between protein intake and the risk of osteo-

porotic fractures, these associations in part, are related to the effect of protein on muscle performance, which is also influenced by many other nutritional and non-nutritional factors. Study of Abelow (130) should be interpreted with caution because many other factors differ between countries could be responsible for the observed association. In the studies by of Munger et al (106) and Feskanich et al (124) no association between animal protein and hip fracture risk was found although in the latter the risk of forearm fracture slightly increased with animal protein. Results of the two other studies indicate that BMD does not differ between vegetarian and omnivorous women (131) and is not associated with the ratio of animal to vegetable protein (132). However, there are important differences between plant and animal proteins; animal foods provide predominantly acid precursors (correlation with urinary acid excretion r = 0.14, $P<0.001$), while plant proteins have substantial amounts of base precursors (correlation with urinary acid excretion r = -0.16, $P<0.001$) (103). Additionally, associations between animal and plant protein with other nutrients follow different patterns; animal protein is positively correlated with dietary calcium (r =0.32, $P<0.001$), saturated fat (r = 0.30, $P<0.001$), and vitamin D (r =0.28, $P< 0.001$) and is negatively correlated with carbohydrate (r =-0.56, $P<0.001$) and polyunsaturated fat (r =-0.24, $P<0.001$). Vegetable protein on the other hand, is correlated with carbohydrate (r =0.44, $P<0.001$), polyunsaturated fat (r =0.17, $P<0.001$) and animal protein(r =-0.43, $P<0.001$) (106). It is also well known that red meat, as a major source of animal protein is a major source of some other nutrients, such as vitamin D, Zn, Iron and phosphorus. Plant protein is also accompanied by some associated nutrients such as vitamin C, A, and K (5). All these nutrients are important in bone metabolism and thus, it is difficult to disentangle the effects of these nutrient from that of protein only. Furthermore, unrecognized non-protein constituents of animal and plant foods may be responsible for the observed differences between animal and vegetable proteins regarding to bone health. However, protein quality (in terms of amino acid constituents) and bioavailability of different proteins may vary according to the source of protein.

2.2.2.7. Summary

In conclusion, current evidence on relations between bone health and dietary protein intake is not consistent. Other than differences in populations and their characteristics, dif-

ferent methodology, sample size and power issues as well as considered confounders may explain some of these discrepancies.

Besides differences in methodology and considered variables, one of the main limitations of most studies might be the failure of disentangle between low protein intake and malnutrition. Such issue is highlighted by the strong correlations between protein intake and the intake of other nutrients, seen in these studies. Subjects with low protein intake are more likely to be undernourished, a condition which may inversely affect their bones as well as other aspects of health. Therefore, it might be of importance to differentiate between malnourished subjects with those who consumed less protein but otherwise are healthy and well-nourished. A reliable method for identifying malnutrition may be essential.

Other limitation of these studies might be their single nutrient approach. In most studies only few nutrients were taken to account and many others were neglected. Analyses based on single nutrients must be interpreted with caution because of the collinearity of nutrient intakes. This fact however, had been noted by several authors, who tried to find some relations between bone health and some ratios such as calcium/protein ratio or animal/vegetable protein ratio. It appears that these ratios are also inadequate to address the multi-collinearity of diet and its relations to a multifactorial condition such as osteoporosis. On the other hand, as stated before, bone is the most protein-dense tissue in the body and hence, it is important to be continuously supplied with new proteins for rebuilding purposes and replacing the degraded amino acids. Therefore, it is not surprising if protein is related to bone health in isolation.

From a physiologic perspective, protein is an essential structural component of the bone, is essential for the production and function of IGF-I (an anabolic factor for bone growth and maintenance) (134), and is a major contributor to the metabolism of calcium and vitamin D. Protein also influences the rate of fall and thereby, the risk of fracture through its detrimental effects on muscle performance and strength.

Associations between dietary protein and bone health may be site-specific, as seen in studies, in which different skeletal sites were examined. For example, while forearm fracture risk was associated with high protein intake, no relation between hip fracture risk and

protein intakes was found (124). It may indicate that local factors are also in operation. Differences in the proportions of cortical and trabecular bone tissues, and the magnitude and direction of the mechanical stresses placed on bone may provide explanation. Trabecular bone tissues are more active and have nearly tenfold higher active surface area than compact bones and, therefore, may be more affected by metabolic variations (17). Mechanical stresses and their directions are also major determinants of bone remodelling and maintenance. Furthermore, associations between protein intake and bone health seems to be conflicted by age and menopausal status in women, possibly because of the huge differences between pre and post menopausal women in terms of hormonal profile.

Another striking feature is the interaction between protein and other nutrients, particularly with calcium. Protein increases urinary calcium excretion. At the same time, it increases the intestinal absorption of calcium and thus, may offset increased excreted calcium (113;114;116). In contrast, Spencer et al (118) and Heaney (117) found no association between protein intake and calcium absorption or calcium balance. However, the effect of protein will not persist for a long time as was shown by Promislow et al (122) and Spencer et al (119). Investigation on calcium/protein ratio revealed inconsistent results (102;105;124;125).

Another implication of protein-nutrient link is the correlations between nutrients and protein. High protein intake correspondingly is accompanied by an increase in other dietary nutrient intakes (49;105;106). Therefore, any nutritional intervention or change in protein intake in the general population or any specified population may affect the intake of other nutrients, which may be important because of other health considerations.

In addition to the correlation between protein and other nutrients, the source of protein appears to play a role. Proteins with different sources may be accompanied by different nutrients and non-nutrient components, which might be unknown for us, while are in operation on bone. An alternative approach might be considering the protein intake in the context of the entire diet and eating habits, in which almost all foods from any source are considered as are eaten in a usual diet. However, the effect of animal protein may be different from that of vegetable protein but the evidence in this regard is mixed.

Beyond these considerations, high protein intake in most studies posed no risk on the skeleton, especially at femoral and vertebral sites (two common sites for osteoporotic fractures). Dietary protein intake was shown to be associated with higher BMD at femoral neck, lumbar spine and radial bone (49;105;120;122). Serum albumin concentration was positively associated with BMC in men (128) and negatively related to the risk of hip fracture in women (135). Protein supplementation was also found to lower the rates of complications after occurrence a fracture, better recovery and shorter length of hospital stay as well as better long-term prognosis for injured subjects (48;126;127). On the other hand, some reports (124;130) but not all (106) are indicating that high protein intake may be associated with increased risk of fractures.

It must be pointed out that the intakes of protein in amounts extremely high or low are obviously incompatible with health. Low protein intake leads to malnutrition, which in turn results in impaired growth and poor bone health (136) and extremely high amounts of protein intake are toxic. Therefore, it is important to investigate whether in a usual diet of a community, upper range of the intake of protein is harmful or not? In this regard Kerstetter et al (115) have suggested that an amount of 0.9 to 1.0g/kg body weight of protein is compatible with a good bone health and Bonjour (127) showed that a supplement, by which protein intake increases only up to the normal level of 0.8 gm/kg body weight is beneficial for bone health. However, the protein must be taken in a normal and otherwise balanced diet to optimized bone maintenance and health.

2.2.3. Vitamin D

Vitamin D is a fat-soluble vitamin with a variety of functions in the body and two major sources: dietary sources and skin synthesis. Its metabolism, absorption, skin synthesis, function, reference measurement and storage were discussed in previous sections. Here, epidemiological evidence of the relationship between bone health and vitamin D situation will be discussed.

2.2.3.1. Vitamin D deficiency and osteoporosis

Gross deficiency of vitamin D in children leads to Rickets and in adults causes osteomalacia and muscle weakness (5) but sub-clinical vitamin D deficiency, which is common in the elderly, appears to be a cause of osteoporosis in advancing age (74).

As cited before, vitamin D nutritional state depends upon two sources: skin synthesis and dietary intakes. With advancing age, both of these sources of vitamin D are limited and less efficient. Dietary supply is limited due to: low vitamin D intake in the diet of older people and low vitamin D absorption in their GI, because of impaired lipid digestion (decline of digestive enzymes), and some degrees of malabsorption (5;137). Cutaneous synthesis of vitamin D is also less efficient in the elderly, because of a decrease in thickness of epidermis, and a decline of 7-dehydrocholestrol in the subcutaneous tissue as well as insufficient sun exposure due to the age-associated frailties (61;137). Consequently, elderly people are at a higher risk of vitamin D deficiency than other groups in the community. Currently, for people aged 65 years or more, the RNI of 10μg/day is advised in the UK (4) and the mean dietary intake of vitamin D in elderly population in the UK is 2.4μg (SD=1.64) (60).

Theoretically, vitamin D deficiency can lead to secondary hyperparathyroidism, which in turn may accelerate bone loss and thus, contribute to the pathogenesis of osteoporosis (5;137). Vitamin D also stimulates the production of IGF-I, which is known to favourite bone mass (55), low vitamin D states can lead to a low production of this anabolic factor.

Published studies suggest that older people are at a higher risk of vitamin D under nutrition, particularly persons who suffering from osteoporosis.

Negative association between age and plasma level of vitamin D was reported by Jaques et al (74) in a study in 759 men and women aged 67-95-y, who participated in the Framingham Heart Study. Dietary vitamin D intake was assessed by a validated FFQ and sunlight exposure was estimated by the place of residence and time spent outdoors as well as the season of examination (method is not exactly described). In this American population sample the mean 25(OH)VitD concentration was 82 nmol/L in men and 71 nmol/L in women. Plasma level of 25(OH)VitD in women was 0.7nmol/L lower for each year of age (P<0.004) but in men age was unrelated to the vitamin D status. Prevalence of vitamin D deficiency (25 nmol/L or less) in this study was 2.4% in men and 4.1% in women. Low vitamin D concentrations (37.5 nmol/L or less) were seen in 6.2% of men and 14.5% of women. Dietary intake and sunlight exposure did equally contribute to the variance in

25(OH)D concentrations, the former being more important in women and the latter in men. However, imprecise measurement of sunlight exposure as an important determinant of vitamin D status was the main limitation of this study.

High prevalence of hypovitaminosis D in elderly ages was also found in another study in a group of elderly Irish people (138). The mean serum 25(OH)VitD in 181 elderly subjects was 10.0 nmol/L (95% CI, < 5.0 to 59nmol/L) without any gender difference. This value was lower than values obtained in 28 young adults (mean = 33.5nmol/L 95% CI 11.5 to 98nmol/L) from the same area. 56% of elderly group had a serum level of 25(OH)VitD below 5 nmol/L and 79% had a value below 25 nmol/L, a level that is considered as vitamin D depletion. Low serum 25(OH) VitD concentrations were noted more in institutionalized subjects than in free-living ones (63% vs. 17% had serum 25(OH)VitD concentrations lower than 5nmol/L and 84% vs. 50% had lower than 25nmol/L). These values were obtained during winter-spring months. At the end of summer from 31 free-living subjects 8 (26%) had plasma level of 25(OH)VitD lower than 25 nmol/L. The mean seasonal increment in serum 25(OH)VitD was also less dominant in elderly subjects (n = 31) than in healthy young adults (n = 8) (24 nmol/L vs. 44 nmol/L).

These two cross-sectional studies showed different prevalence rates of hypovitaminosis D that maybe due to differences in environmental variables such as latitude and ultraviolet exposure rate as well as differences in dietary intake and fortification of certain foods in their communities. The effect of seasonal variation is also important. However, in the second study comparison between young adults and elderly subjects was helpful to show higher prevalence of hypovitaminosis D in old persons.

Negative correlation between plasma level of vitamin D and age was found also in another study by Orwoll et al (128), stated before (see section 2.2.2.4). In this study plasma level of all three metabolites of vitamin D (25(OH) VitD,1,25$(OH)_2$VitDand 24,25$(OH)_2$VitD) were negatively associated with age (P<0.01).

Omdahl et al (139) also reported similar results from their investigations on 304 healthy free-living elderly people (166 women and 138 men) over 60-y of age. They found that 48% of men and 54% of women consumed less than RDA (10 μg for USA population) for

vitamin D and also 49% of men and 67% of women consumed less than RDA of 800mg/d for calcium in American population. Dietary calcium intake was associated with dietary intake of vitamin D (r = 0.54, $P<0.0001$). When compared with a young adult group as controls (n = 47 mean age 32-y), the older population had significantly lower plasma level of 25(OH)VitD (15.5 ± 7.2ng/ml vs.29.1± 9.7 $P<0.0001$).

Hypovitaminosis D is more common in osteoporotic-fractured patients than in healthy elderly people. This was shown in three case-control studies, which are summarized in Table 2.3.

Table 2. 3- Vitamin D status in osteoporotic vs. non-osteoporotic subjects.

	Lips et al (140)		Baker et al (141)		Diamond, et al (142)	
	Cases**	Controls	Cases	Controls	Cases	Controls
n	125	74	98	76	41	82
Age	75	75	65	65	>65	>65
% of vitamin D deficiency	62%	16%	40%	17%	63%*	25%*
Serum level of 25(OH) VitD (nmol/L)	18.5	32.9	34	56	46	61
Vitamin D dietary intake (IU/d)†	115	115	97	118		

* Vitamin D deficiency in this study was defined as serum level of 25(OH) VitD <50 nmol/L.
**Cases were hip fracture patients.
†40 IU=1μg

As Table2.3. shows all studies indicate lower vitamin D status in hip fracture patients compared to controls. Higher prevalence of vitamin D deficiency, define by serum 25(OH) VitD indicate that hip fracture patients are more likely to be vitamin D deficient.

2.2.3.2. Seasonal variation

Several studies have reported higher levels of vitamin D in summer than in winter. This seasonal variation may contribute to the osteoporosis in the elderly (4). Storm et al (143) have reported a decline of about 20% in 25(OH) VitD concentration in winter compared with summer ($P = 0.001$) and conversely, 20% increase in plasma PTH in winter among a sample of postmenopausal women (n = 60). Bouillon et al (144) on a sample of 240 elderly subjects from Belgium also reported the lowest plasma level of 25(OH) VitD from February-May (mean level less than 25nmol/L) and the lowest level of free and total $1,25(OH)_2$Vit Din February- March (50± 40 pmol/L), peak values in summer were more than two times as winter.

Jacques et al (74) have also reported similar seasonal variation in their study on 759 elderly men and women from USA. Among women, during winter and spring, serum 25(OH) VitD were 14.8 nmol/L(P<0.001) and 8.9 nmol/L (P= 0.007) lower than summer, respectively. Corresponding values among men were 30.1 and 21.0 lower than summer values (P<0.001). In all seasons, differences between serum level of 25(OH) VitD in men and women were significant except for winter, which was –1.4 nmol/L (P=0.79). Dawson-Hughes et al in a study on 391 men and women aged over 60 showed that during wintertime (February-May) more than 90% of men and women had 25(OH) VitD concentrations lower than 110 nmol/L, a value at which plasma level of PTH began to increase. Differences between men and women diminished in winter, while, in other seasons men had higher concentrations of 25(OH) VitD than women.

Seasonal variation has also been found in subjects who were not exposed to sunlight. Devgun and colleagues (145) compared three groups of subjects; outdoor workers (n = 18), who were exposed to sunlight 6-8h/day, indoor workers (n = 8), who were expose to sunlight mainly at weekends and evenings and long-term inpatients (n = 7), who did not access to artificial or natural sunlight during study period. Seasonal variations were seen in all three groups but in different months. Peak of 25 (OH) VitD concentrations was seen in November, October and August, respectively. Peak of ultraviolet radiation was in July. The peak value of 25(OH) VitD was highest in the first group and lowest in the third group, which were related to outdoors spending time. Similar variation has been reported by others (146).

In summary, all studies on vitamin D determinants have confirmed the effect of seasonal variation but it varied according to the latitude and outdoors spending time. Magnitude of such variation is conversely related to BMI and age (61;147). Seasonal variation in vitamin D status among subjects who were not exposed to sunlight maybe due to a similar variation in vitamin D contents of certain foods during high ultraviolet exposure months (145). It is also more dominant in men than in women, possibly because of higher plasma level of vitamin D and higher outdoors spending time in men (74;147) in comparison with women. However, it has been suggested that to maintain plasma levels of 25(OH) VitD

above 15-22.5nmol/L in winter, during summer month it should be kept at levels greater than 40nmol/L (63).

2.2.3.3. Gene–environment interactions and the effect of vitamin D

A sometimes ignored feature of the vitamin D function is the intermediary effect of vitamin D receptors (VDR). It is important to distinguish between the plasma level of vitamin D and its effects on the target tissues, which is mediated by its receptors. These receptors are genetically regulated and their variation is believed to be responsible for 7-10% of the difference in bone density in postmenopausal women (75). Association between these receptors and bone mass still remained controversial. A twin study in Britain showed a strong influence of the vitamin D receptor genotype on bone density at various skeletal sites (75) 95 dizygotic (non-identical) and 87 monozygotic (identical) pairs of twins aged 50-69 years were participated in this study and BMD was measured at the hip, lumbar spine, forearm and the whole body. From differences in associations between BMD measures in concordant (those with identical genotype) and the discordant pairs (those with differing genotype) at all sites, authors concluded that the VDR genotype contributes to the genetic regulation of bone mineral density. Age, weight, years since menopause, and use of HRT were taken to consideration. Although the study had a number of limitations such as considering a few bone affecting variables including nutrition, neglecting the modification influence of early life exposures, which is very likely to be different in twin studies, the results are helpful to explain, in some part, the discrepancies in the studies of diet and bone health. It may also be of benefit for identifying persons who are genetically at higher risk of osteoporosis and target them in prevention policies.

Significant association between VDR genotypes and spinal BMD was also reported by Tamai et al (76) in Japanese women. Spinal BMD in three groups of women with osteoporosis (n = 90), osteoarthrosis (n = 36) and healthy subjects (n = 92) was assessed by DXA and their VDR were genotyped. Although the frequencies of three genotypes ("bb", "Bb" and "BB") were not significantly different among three groups of subjects, healthy subjects with VDR genotype of bb had significantly higher age-matched BMD than that of subject with "Bb" genotype ($P<0.03$). In contrast, osteoporotic "BB" patients had higher age-matched BMD in comparison with "Bb" genotyped subject ($P<0.02$). There

was no difference by VDR genotype in spinal BMD in subject with osteoarthrosis. This study again, failed to consider other bone-affecting variables, even nutritional ones, which may have modified the associations between bone measurements and genetical factors (148;149). Other studies however, reported no association between VDR genotype and bone mineral measurements (78;150;151). Laskey et al (151) found no relation between VDR genotype and changes BMD of the whole body, spine, hip, and forearm among breast-feeding mothers. Arden et al (78) found also no relations between calcaneal ultrasound measurement and VDR polymorphism in 189 pairs of healthy dizygous twin females.

There is evidence indicating that the relations between VDR genotype and bone health is subject to modification by current and early nutritional exposures. Cooper et al (150) found significant association between VDR genotype and weight at one year (7% higher 1-y weight in homozygote "tt" in comparison with homozygote "TT") ($P = 0.04$) in 66 elderly females. However, they found no association between VDR genotype and BMC or BMD at the lumbar spine and femoral neck.

A recent investigation in this regard showed that intrauterine undernutrition may modify VDR genetical influences on bone (73). BMD at lumbar spine and proximal femur of 165 men and 126 women aged 60–75 years were assessed at baseline and 4 years later by DXA and their VDR genotypes were determined by standard procedures. Although VDR genotypes and birthweight had no association with bone measurements, associations between VDR genotypes and BMD at both sites varied according to birthweight. Among subjects in the lowest third of birthweight, those with the "BB" genotype had higher spinal BMD ($P = 0.01$), while among those in the highest birthweight "bb" genotype accompanied by a higher spinal BMD ($P = 0.04$). Similar associations were found between spinal BMC and VDR genotypes in low-birthweight group. Associations were adjusted for age, sex and weight at baseline. Associations between BMD and BMC at femur with VDR genotypes did not reach to a statistically significant level.

The effect of VDR genotype on bone has been shown to be modified by current nutritional intakes (148;149). Graafmans et al (149) investigated the influence of VDR genotype on rate of bone gain in 81 women aged 70 years and over who had taken part in a

placebo-controlled vitamin D supplementation trial (400 IU daily for at least 2 years). Femoral BMD changes were examined in relation to VDR genotype over the course of 2 years. Authors showed a more pronounced effect of vitamin D supplementation among subject with "BB" genotype compared with those with the "bb" genotype on femoral BMD ($P = 0.03$).

Similar to this study, Krall et al (148) evaluated the influence of VDR genotype on rates of bone loss in 229 elderly postmenopausal women who had participated in a placebo-controlled calcium supplementation trial (500-mg/day calcium supplement). BMD was measured over a period of 2 years at the femoral neck, spine, and radius. Analyzing the study population according to their calcium intake level revealed that VDR genotype influenced bone loss at femoral site only in those with low calcium intake. In this study however, rates of bone loss were greater in the "BB" group at all sites.

In summary, in spite of disagreements seen between the published studies about the genetic links between vitamin D activity and bone health, these studies provide valuable information for research purposes and preventive strategies for osteoporosis. Although our knowledge still appears to be inadequate to depict a comprehensive model of genetic-environmental interactions, it may have pointed to novel aspects of early and late nutritional exposures and their modifications on genetical determinants of osteoporosis. However, because most studies on VDR genotype suffered from limitation of considering many bone-affecting variables, and in particular, nutritional ones, the results must be interpreted with caution. More comprehensive investigations are obviously needed.

2.2.3.4. Summary

In conclusion, all above cited studies have shown higher prevalence of hypovitaminosis D in elderly ages particularly in subjects who suffered from osteoporotic fractures and low sunshine exposure is an important determinant contributing to this propensity. In this regard, the effect of seasonal variation, physiologic limitation of skin synthesis of vitamin D, and the effect of environmental factors, which may influence ultraviolet exposure, are important and might be a source of variation in vitamin D state and osteoporosis. Disentangle between physical activity, which is a bone-affecting factor and mostly is related to outdoors spending time, and sunlight exposure is still problematic. In addition, association

between sunlight exposure and vitamin D status is influenced by other factors such as age, sex and BMI. Age and BMI are negatively associated with vitamin D status and peak of vitamin D obtaining in the summer (74). This effect of BMI is in contrary with positive effect of weight on bone mass, which is described in the next sections.

Both carrier (DBP) and cellular receptor of vitamin D (VDR) are important in its function and both are regulated mainly by genetical factors, although exposures of early life in utero and infancy may modify their function.

Interactions between vitamin D and other nutrients are also of particular importance. Many nutrients can influence the function of vitamin D on bone and other tissues through their effects on DBP and VDR. Also, increasing vitamin D intake directly will increase intakes of other nutrients such as energy, protein and calcium, which are important in bone metabolism. So, it seems more appropriate to consider vitamin D status in the context of other nutrient intakes.

Apart from effects on bone metabolism, vitamin D is determinative for the function of muscles, which is closely related to the risk of fall and osteoporotic fractures. These interactions are discussed under related headings (see section 2.7)

2.2.4. Calcium

Calcium as the main structural bone element has been the nutritional focus of osteoporosis research over recent decades, although the results have been controversial. Many studies reported salutary effects (125;152-156), some others reported no association (157-160) and a few studies found adverse relation between bone health and calcium intake (161). Issues related to the absorption and dietary sources of calcium discussed in chapter one (section 1.4.5.1). Epidemiological evidence on the relationships between calcium intake and bone health, particularly in the elderly, will be reviewed in this section.

Table 2.4. presents the key findings and main features of studies of bone health and calcium intake. One of the early prospective studies (152;153) from California found that from the highest third of dietary calcium (> 440 mg/1000 kcal) to the lowest two thirds of calcium intake (0-440mg/1000kcal), the risk of hip fracture decreased to 0.3 for men and to 0.4 for women (95% CI is not mentioned) ($P<0.05$). These associations remained un-

changed after adjustment for age, sex, BMI, alcohol, smoking, and HRT (hormone replacement therapy). Calcium intake was lower in hip fractured patients in comparison with those without fracture. Adjusted intakes of protein, vitamin D, magnesium and phosphate were significantly higher in high calcium intake group than in the other groups. In this study however, dietary assessment was based on 24-hour dietary method, which may not represent a usual diet, even though adjustment for age and energy intake may have reduced the effect of within-individual variation. Furthermore, evaluation of HRT was not precise and the cohort maybe unrepresentative because of the sampling method.

Table 2. 4 – Studies of calcium intake and bone health.

Author	Subjects			Study design	Dietary Ass	Outcome measurement	Results
	n	Sex	Age(y)				
Holbrook et al (152)	957	M,F	50-79	Cohort (14-y)	24-h recall	Hip fracture risk	Decreasing the risk of hip fracture by increasing calcium intake.
Lau et al (155)	1200	M,F	<70->80	Case control	Weekly-diet history	Hip fracture risk	Decreasing the risk of hip fracture by increasing calcium intake.
Picard et al (156)	183	F	40-50	Cross-sectional	3-day recall	BMC of spine and forearm	Positive association.
Metz et al (125)	38	F	24-28	Cross-sectional	FFQ	BMD and BMC of radial bone	Positive association.
Devine et al (154)	124	F	PM*	Cohort (2-y)	4-day diet record, 3 times during the study	BMD changes at hip, spine and ankle.	Positive association.
Looker et al (158)	4342	M,F	50-74	Cohort (16-y)	24-h recall	Hip fracture risk	No association.
Owusu et al(160)	43,063	M	40-75	Cohort (8-y)	FFQ	Hip and forearm fracture risk.	No association
Tavani et al (157)	960	M	PM, >45	Case control	FFQ	Hip fracture risk	No association
Cooper et al (159)	900	M,F	<55->84	Case control	FFQ	Hip fracture risk	No association
Kreiger et al (161)			50-84	Case control	FFQ	Hip and wrist fracture risk.	No association on hip fracture but decrease wrist fracture risk

M =Male, F = Female

A further study by Lau et al (155) from Hong Kong showed that men with lowest calcium intake (<75mg/d) in comparison with those of the highest calcium intake (244mg/d or more), were at a twofold risk of hip fracture (RR= 2.1, 95% CI 1.1-4.2), comparable RR for women was 1.9 (95% CI, 1.2-2.9). The mean daily calcium intake of cases was lower than that of controls (in females: 128mg/d in cases vs. 168 mg/d in controls, and in males: 141 mg/d in cases and 177 mg/d in controls). Mean calcium intake in the study population was 171mg/d.

Picard et al (156) showed a significant favourable effect of calcium intake on bone mass at the lumbar spine and distal forearm after being controlled for parity, physical activity, oral contraceptive use, smoking, height and weight. However, because of selected age criteria, the effect of menopause on bone mass has not been examined and the results could not be extrapolated to postmenopausal women.

Metz et al (125) have also reported a beneficial effect of calcium intake on BMD and BMC of radial bone at the middle and distal sites. Subjects in this study, however, were young adults and, therefore, the results may not be applicable to the elderly population.

In accordance with these studies, Devine et al (154) reported a favourable effect of calcium on bone mass in postmenopausal women, who had no menstruation for >10 years. From the pattern of changes in BMD over 2 years, the authors concluded that bone loss at the femoral neck might be prevented by daily calcium intake of 2042mg/day (51 mmol/day). This amount varied for other sites.

Some other studies reported no association between calcium intake and bone health (157-160). Looker et al (158) reported a RR of 0.5 (95% CI 0.2-1.2) for hip fracture in postmenopausal women and older men after controlling for HRT, smoking, physical activity, BMI and alcohol use. Hip fractured patients in this study did not differ from non-fractured subjects in terms of daily calcium intake and calcium/1000kcal. The study power was low (40% in men and 50% in women to detect a RR of 0.5) and few variables and nutrients were considered in the analyses. The dietary assessment (24-h recall questionnaire, at baseline) may not represent usual diet. Note that the mean calcium intake in the whole study population was 658mg/d.

Owusu, et al (160) have also reported no association between total calcium intake and fracture incidence. Age, smoking, physical activity, alcohol consumption, BMI and total energy intake were considered for adjusting the RRs of hip and forearm fracture in this study. Calcium consumption, either from dairy or non-dairy sources, was not associated with fracture risk. However, the number of hip fractures in this study was small (201 fore-arm and 56hip fracture).

Similarly, Tavani et al (157) also showed a non-significant association between hip fracture in women and calcium intake. There was also no association between milk or cheese consumption and hip fracture.

A case-control study in Britain (159) also showed no association between calcium intake and hip fracture in women but a decreased risk in men who had a calcium intake above 1g/day. Separate analyses for men and women showed different patterns of osteoporosis. The risk of hip fracture among women did not change with increasing calcium intake, but in men there was a fall in risk with increasing calcium intake. Adjusting for BMI, smoking, stroke, alcohol and steroid treatment changed this trend. Adjusted RRs for women showed small differences across fifths of distribution (of calcium intake) and adjusted RR among men with the highest intakes, more than 1041mg/d, remained significantly lower than the rest. Mean calcium intake in this study was 689mg/d.

Kreiger et al (161) reported a contra-directional effect of dietary calcium on hip and wrist fractures. While it increased slightly the risk of hip fracture, wrist fracture risk was significantly decreased by calcium intake of 1gm/day or more. OR for hip fracture in highest third of calcium intake (>1000 mg/day) comparing with the lowest one (<800mg/day) was 1.89, (95% CI, 0.75-4.74). Comparable OR for wrist fracture was 0.18, (95% CI 0.04-0.81). Alcohol, smoking and reproductive activity were considered, but physical activity was not evaluated in this study.

The results of presented studies are not consistent. Differences in methodology, considered variables, and characteristics of the populations under study might be accounted for the discrepant results. Most discrepancies were among investigations, which were concerned to the rate of osteoporotic fractures. Studies on relations between calcium intake

and bone mass, on the other hand, were generally more consistent, showing positive association between bone mass and calcium intake (125;154;156). Studies on fracture risk and calcium intake produced inconsistent results (152;153;155;157-161;161). Although bone mass is a major determinant of fracture (7), its affective variables are somewhat different from those of fractures. Fracture is related to bone strength in one hand and the risk of fall on the other, and each, is under influence of different factors (162). Furthermore, small changes in BMD or BMC may be statistically significant, but it is not clear whether such changes have a significant effect on the fracture rate.

However, studies of the effect of calcium supplements on BMD and BMC appear to be helpful for more clarification about the effect of calcium on bone. Next section is dedicated to this issue.

2.2.4.1. Supplementation trials

In the study of supplementation trials, it is important to note that many of the supplementation studies had administered vitamin D simultaneously with calcium. Administration of vitamin D supplements alone has been reported to inhibit bone loss and, therefore, it is difficult to disentangle the effect of calcium supplements from that of vitamin D. Additionally, calcium supplements are usually calcium salts, containing remarkable amounts of base precursors, which are shown to be effective on calcium metabolism and, therefore, the effect may be different from that of dietary calcium (163).

Table 2.5 presents studies of calcium supplementation and bone health. Most of the studies were carried out in postmenopausal women and frequently found protective effects. One from New Zealand (164) found a less bone loss at the femoral, total body and lumbar spine in supplemented group compared to that of placebo group. In this study, the effect of supplementation on divergence the rate of bone loss between the two groups sustained during the course of 4 years for the total body and lumbar spine with the exception of proximal femur, where after two years no further divergence had been occurred. However, at any given time the BMD of the femur was significantly higher in supplemented group than in placebo group. Differences between groups were significant for all BMD measures. Median dietary calcium intake for the whole group was 700mg/d at baseline and subjects on average were 9 years postmenopausal.

Table 2. 5- Studies of calcium supplementation and bone health.

Author	Subjects			Duration (y)	Supplement		Outcome measure	Results
	n	Sex	Age		Calcium	Vitamin D		
Reid et al (164)	86	F	58	2-4	1g/d	-	BMD changes of femur, spine and total body	Favourable effect
Reid et al (165)	122	F	PM>3 y	2	1g/d	-	BMD changes of femur, spine and total body	Favourable effect
Storm et al (143)	60	F	>65	2	1g/d	-	BMD changes of femur and spine	Favourable effect
Chapuy et al(167)	3270	F	84	3	1.2g/d	800IU/d†	Hip fracture risk	Favourable effect
Lee et al (168)	20	F	70	0.5	0.71g/d	399 IU/d†	Hand BMD	Favourable effect*
Baeks-gaard et al (169)	240	F	58-67	2	1g/d	560 IU/d†	BMD changes of hip, spine and fore-arm	Favourable effect at spine, no effect for hip and fore-arm.
Chapuy et al (170)	297	M,F	74-83	0.5	1g/d	800 IU/d†	Plasma calcium, vitamin D, ALP and PTH level	Favourable effect
Riis et al (171)	43	F	50	2	2g/d	-	BMC changes of spine, total body and forearm	No association
Dawson-Hughes et al (172)	301	F		2	0.5	-	BMD change of femur, spine and radius	Beneficial effect in those with low calcium intake and more than 5-y menopausal.

†40 IU=1μg

Storm et al (143) in a supplementation trial compared two methods of supplementation: dietary means (with milk) and calcium salts. In this study 60 postmenopausal women without osteoporosis were randomized into three groups: 1) dietary milk supplementation (four glasses milk, daily), 2) calcium carbonate supplementation (1000mg/d) and 3) placebo group. After two years supplementation, bone loss at greater trochanter was 3% ($p<0.03$) in controls, 1.5% ($P = 0.03$) in dietary supplemented group and no bone loss was observed in calcium carbonate supplemented group. Calcium carbonate supplemented group also showed a significant increase in BMD at spinal and femoral neck ($P<0.05$). This study showed a significant effect of calcium supplementation on bone density of

postmenopausal women. Total calcium intake in placebo group was 683mg/d and in dietary supplemented group was 1028 mg/d and for women who received carbonate supplementation was 1633mg/d.

Chapuy et al (167) reported an odds ratio of 0.73 (95% CI, 0.62-0.84) for hip fractures and 0.72 (95% CI, 0.60-0.84) for all non-vertebral fractures.

Lee published another supplementation trial (168), in which individuals were supplied with calcium rich food (cheese), calcium phosphate (350 mg calcium and 270mg phosphate per day) and vitamin D (399 IU/d = 10μg/d), for a course of 6 months. Bone density at hand (measured at 3 phalanx sites) increased in 11 subjects ($P<0.05$) with no changes in three and reducing in six subjects. Dietary calcium intake before supplementation was 452mg/d (±191) and calcium intakes from supplements were 710mg/d. These results indicate the favourable effect of calcium supplementation in elderly people, although some other variables might be also in operation.

Baeksgaard et al (169) reported and increase in BMD at lumbar spine of 1.6% during 2 years in treatment group ($P<0.002$) but not in the placebo group. No significant changes from baseline value were observed at the distal forearm and hip. Mean intake of calcium and vitamin D in this population were 919 mg/d and 3.8 μg/d, respectively.

Chapuy et al (170) showed an increase of 67 μmol/L in the plasma level of calcium and of 30nmol/L 25(OH)VitD from baseline among treatment group ($P<0.02$), respectively. In contrast, calcium concentration decreased in controls (mean = -25±110μmol/L) and 25(OH) VitD increased less than treatment group (19 nmol/L).The mean $1,25(OH)_2$Vit D value did not change in treated and control group. The mean changes in values for 25(OH)VitD from baseline in treated and untreated subjects were +38 and +1nmol/L, respectively ($P< 0.001$). PTH levels and alkaline phosphatase levels reduced in treated groups in comparison with controls and were negatively associated with increments in plasma 25(OH)VitD ($r = 0.41$ $P<0.001$) and calcium supplementation ($r =0.331$, $P =0.01$). The mean dietary intakes of calcium and vitamin D for the whole population were 367mg/d and <5μg/d, respectively, and 62% of the subjects had low dietary calcium intakes (<500mg/d). No evaluation for the effect of supplementation on bone health has

been done. However, plasma $1,25(OH)_2$Vit D is also under physiologic controls and lack of changes in its concentration does not indicate any effect of supplementation.

Riis et al (171) reported that BMC at forearm, the total body and spine fell significantly in both placebo and treatment group but remained constant in subjects who received HRT. Small differences between placebo group and calcium-supplemented group were not significant for the total body, distal forearm and spine. At the proximal forearm, although BMC was significantly higher in supplemented than in placebo group, bone loss still was apparent in both groups. These results indicate that supplementation with calcium might not be of benefit in early postmenopausal women.

Dawson-Hughes et al (172) found that the effect of calcium supplement on bone health in postmenopausal women is influenced by calcium intake and menopausal status. Among early postmenopausal women (>5 years after menopause), changes of BMD at all measured skeletal sites over two yeas supplementation were not significantly different between treatment and placebo groups, in spite of significant increase in the plasma values of 25(OH) VitD, calcium and 24-h urinary calcium excretion. Among late postmenopausal women on the other hand, supplement beneficially affected the BMD changes in the group of lower dietary calcium intake (>400mg/d), with no statistically significant effects in those with higher calcium intakes (400-650mg/d). A number of life style variables, smoking and alcohol intakes were taken to consideration in this study.

In summary, the positive effect of calcium supplementation was reported by almost all presented studies. Differences between studies with respect of the age of participants, calcium intakes, sex and years after menopause provided important clues for the effect of calcium on bone. These effects are comparable to the associations seen between dietary calcium and bone health. Effect of calcium supplementation was shown to be site-specific. More pronounced and more sustained effects were shown at the lumbar spine, where the composition of bone is predominantly trabecular, than femoral or radial bone sites, where the composition of bone is mainly cortical (164;169). This effect may be due to higher metabolic activity of the trabecular bone tissues in comparison with cortical bones.

In most studies, supplementation was given for a relatively short term of 1 to 2 years, with only one study continued for up to 4 years. This period may be inadequate to assess the long-term benefits of calcium supplements on bone. An observed rise in bone mineral measure after supplementation might be due to a phenomenon known as the "bone remodelling transient" rather than to an actual increase in bone mass (504). Bone remodelling is a process, by which bone is first absorbed by osteoclasts and then rebuild by osteoblasts (see chapter 2). As stated earlier, it takes many weeks or months for the entire process to be completed. Supplementation may lead to a transient decrease in the activation frequency of osteoclasts at the start of the remodelling process. This decreases the proportion of skeletal surfaces that are actively undergoing bone remodeling at any one time, resulting in an increase in the mineral present per unit volume of bone. These alterations in bone mineral can be sufficiently large to be detected by absorptiometry. Bone mineral continues to rise for some time until a new steady state is achieved because of the time lag between resorption and completion of the formation phase (497). Thus the benefit would be of short duration (equivalent to the period of one remodelling cycle) and likely to be fully reversed at the end of the intervention period. Furthermore, because of coupling within each bone remodelling cycle, decrease of bone remodelling ultimately might reduce the bone formation rate (497). However, whether a reduced rate of skeletal remodeling represents a benefit for bone health in elderly is unknown. Although reducing bone turnover might be beneficial (173), it could not prevent age-related bone loss for a long time, especially in cortical bones (164).

Baseline calcium intakes appear to play a role in the effects of calcium supplementation, although there was no agreement on a cut-off point in this regard Dawson-Hughes et al (172) found that subjects with an average dietary calcium intake of more than 400mg/d at baseline had little benefit from supplements while others reported beneficial effects of calcium supplementation even in those with fairly good calcium intake at baseline (169). However, in most studies supplementation led to calcium intakes of more than 1000mg/d and its salutary effect was still apparent.

Two studies demonstrated that the number of years after menopause was important in determining the effect of calcium on bone loss (171;172). During this period, the skeleton is

influenced by a huge deterioration of sex hormones and, therefore, the priority in causal pathway is of hormonal changes rather than dietary variation. This priority was shown by Riis et al (171), who found that the effect of HRT on bone in early menopause is much stronger than that of calcium supplementation.

It is noteworthy that the effect of calcium supplementation may not be due to its calcium contents, solely, but other elements may exert some effects; such as base precursors of calcium citrate and calcium carbonate. These components may also exert a pharmacological effect, which is different from that of physiologic one (effect of dietary calcium intake come from a usual diet) (174).

In conclusion, supplementation trials are indicating that the positive association between calcium intake (either through diet or in the form of calcium supplements) and bone health may vary by the skeletal site, the number of years from menopause, the calcium intake level and sex, with higher effects on trabecular bones, in late menopausal women consuming lower amounts of dietary calcium.

2.2.4.2. Summary

Studies that have examined the association between dietary calcium intake and bone health produced inconsistent results. These differences could be attributed to other factors, such as different methods, or differences between populations under study as well as variables that were taken into account. Despite these inevitable differences, there appears to be sufficient evidence to allow the conclusion that calcium intake is determinant for bone health. There is no need to be emphasized that calcium is the most important mineral constituent of the bone and is important for the regulation of PTH and many intracellular enzymatic activities, which are directly influencing bone metabolism and maintenance. Hence, from a physiologic perspective, calcium plays a central role in the metabolism of bone and in the pathology of osteoporosis. The body of epidemiological evidence is also massive; most studies on the relations between bone mass (BMD and BMC) have shown positive relationship between calcium intake and bone health among subjects within different age groups: children (3-10 y) (175) young women (24-28 y) (125), premenopausal (age 40 to 50 years)(156) and postmenopausal women (154). These results are consistent with those from the meta-analysis studies (176;177).

A number of studies that have examined the relationship between calcium and fracture rate also revealed protective effects (152;153;155;178) while others failed to find such relationship (160;179-183). Regardless of the limitations of the studies, differences in the study populations may provide clues. One issue that may be of importance is the difference in calcium intakes; studies on populations with higher calcium intakes found no relation (158;161;180), but studies in population with low calcium intakes reported lower fracture rates with increasing calcium intakes (152;155). For example; two case-control studies with the same method have reported contrasting results; one from United Kingdom (159) with the mean calcium intake of 689 mg/d found no relation between calcium intake and fracture rate in women whilst the other from Hong Kong (155) in a population with mean calcium intake of 171mg/d found protective effect. Also, in the study of Hannan et al (180), in which the mean calcium intake was 800mg/d, and most studies from USA, where calcium intake is generally high, have reported no relation (160;179;180). This is supporting the threshold behavior of calcium. Threshold is the level of calcium beyond which an increase in calcium intake is no longer associated with skeletal accumulation of calcium and below which it varies with variation in calcium intakes. A plausible explanation for that is the physiologic behaviour of calcium; as is stated by Heaney (184), calcium is a "threshold nutrient" because its excess cannot be stored in the body, unless an earlier depletion has occurred. It means that at the balance level, excess of consumed calcium will be excreted from the body, with no further benefit for the skeleton. The threshold behavior pattern of calcium intake has been demonstrated in prepubertal girls (185), and in late postmenopausal women (172). Such behavior also has been documented by published meta-analyses and review papers (176;186;187). However, the threshold value for calcium is the matter of controversy. For elderly subjects, Heany has estimated a level of 1400-1700 mg/d, and for adults a value between 800-1200mg/d (184). Levels lower than 800-900 mg/d have been suggested to be insufficient for optimal bone mass in prepubertal girls (185) and levels lower than 400 mg/d in postmenopausal women were associated with higher bone loss (172). For adolescents, Bonjour reported a threshold level of 1100-1200mg/d (185) Heaney suggested a level of 1350-1550 mg/d (184), and Matkovic suggested a level of 1200-1500 (187). This controversy is not surprising but problematic. The major underlying mechanism in determining this threshold level is adaptation mecha-

nism, which in turn, is influenced by vitamin D status (85), age and early nutritional exposures (188). Racial and genetical factors are also of importance in regulating calcium metabolism and calcium requirements for bone health (189). The interactions of these factors make it difficult or impossible to identify a single unique number for the calcium threshold and requirement for all nations. Therefore, existing different threshold levels for various populations are expected but is essential when the results of studies are compared. The threshold behavior of calcium allowed estimation of calcium requirements and thus is of importance for making nutritional policies. It is worth noting that calcium intakes beyond the threshold level not only has no benefit but also may be threatening because of other health considerations such as: interfering with iron intake, which by itself is a health burden across the world (88).

However, effective calcium intake depends upon many factors other than dietary calcium content, such as nutrient-nutrients, drug-nutrient and disease-nutrient interactions, which may affect calcium bioavailability (83;153;190). Effects of calcium are also conditional upon the efficiency of other nutrients such as vitamin D and protein (see previous sections about protein and vitamin D). Thus, considering just calcium content of a diet neither properly estimates the amount of absorbed calcium nor its effects. Therefore, determining calcium threshold level or calcium requirements is not possible unless other nutritional variables are taken to consideration. Obviously, non-nutritional variables, which may affect bone health, are also of importance.

The effect of dietary calcium intake on bone mass and fracture risk varies between men and women and maybe affected by sexual hormones as well as age and skeletal site. Among women it is less effective in early years after menopause (158) possibly because of the effect of dramatic hormonal deterioration, seen in these women. Bone metabolism in these women is affected mainly by huge variation in sexual hormones rather than dietary calcium intake.

2.2.5. Vitamin K

Vitamin K is a fat-soluble compound with two natural types: phylloquinone (vitamin K_1) from plants and the menaquinones (vitamin K_2) found in fish oils and meats. The latter

could be made by bacteria in the human intestine. Proportional contribution of each form to daily intake is not clearly defined (82).

2.2.5.1. Reference measurements

A blood clotting test, namely, prothrombin time is traditionally used as an indicator of vitamin K status and is the basis for safe intake of 1 μg/kg/d. Recently the plasma concentration of vitamin K and undercarboxylated osteocalcin or undercarboxylated prothrombin have also been used. Plasma vitamin K levels reflect relatively recent vitamin K consumption, while undercarboxylated prothrombin and undercarboxylated osteocalcin reflect the long-term vitamin K status (5;58;82).

2.2.5.2. Vitamin K and osteoporosis

Relationships between vitamin K and bone health have been noted in several studies. One prospective study by Feskanich et al (191) followed-up 72327 women aged 38-63 for 10 years to evaluate the association between vitamin K nutritional status and hip fracture risk. Analyses based on the baseline dietary measurement showed that women with vitamin K intakes of quintiles 2-5 (109 to more than 242 μg/d) had a significantly lower adjusted risk for hip fracture (RR = 0.70, 95% CI 0.53-0.93) compared with those within the lowest quintile 1(<109 μg/d). Women within quintiles 2-5 of vitamin K intakes (109 to more than 242 μg/d) had a significantly lower adjusted risk for hip fracture (RR = 0.70, 95% CI 0.53-0.93) compared with the women of quintile 1(<109 μg/d). There was no difference between quintiles 2 and 5 (Q_2= 109-145 and Q_5 = more than 242 μg/d). No linear dose-dependent response was observed (P = 0.32). These results indicated a possibility of a threshold effect for vitamin K. Adjustment for BMI, menopausal status, smoking, HRT, physical activity and daily intakes of protein, vitamin D, calcium and alcohol did not change the risks substantially. Separate analyses for postmenopausal women did not show any difference for RRs.

Similar results were reported by Booth et al (192) in a study of 888 participants in Framingham cohort (mean age 75). In this study, there was no association between dietary vitamin K and BMD at the hip, spine, and arm. Meanwhile, the risk of hip fracture decreased with increasing vitamin K intake, significantly. Over 7-y of follow-up, individuals

with the highest quartile of vitamin K intake (median: 254 μg/d) were at lower risk of hip fracture (RR: 0.35; 95% CI: 0.13-0.94) than did those in the lowest quartile of intake (median: 56 μg/d).

Findings of another study confirmed the abovementioned results; Hodges et al (193) compared the plasma level of vitamin K in 51 hip fractured patients (mean age of 81 y) with a group of 38 healthy age-matched subjects as controls from the same population. In this study plasma level of vitamin K_1, measured in few hours after hip fracture in cases, was significantly lower than controls (336±302 versus 585 ± 490 pg/ml P<0.00). Plasma concentrations of vitamin K_2 were also lower in cases than controls (89 ± 113 vs. 161 ±145 pg/ml, P<0.01 for menaquinone-8 and 120±84 vs. 226±178 P<0.001 for menaquinone-7).

Supplementation with vitamin K has been shown to reduce under-carboxylated osteocalcin, a marker of bone turnover (194). Two groups of healthy subjects, who had normal coagulation variables, with a total number of 219 (aged 18–30 y and ≥65 y) were enrolled in a supplementation trial of phylloquinone (1000 μg) or placebo for 2 wk. Under-carboxylated osteocalcin decreased by the first week 7.6% to 3.4% (P < 0.001) and sustained over the course of the study. Supplementation was also associated with a significant 10-fold increase in plasma level of vitamin K by week one and continued over the study course. Other markers of bone turnover (bone-specific alkaline phosphatase, *N*-telopeptides of type I collagen) however did not change by supplementation.

Vitamin K antagonists are compounds, which may affect vitamin K state adversely and, therefore, are supposed to affect bone health. Rosen et al (195) published a study in this regard, with two parts: in the first part 50 patients (aged 41-85 y, 20 men and 30 women) with different cardiovascular diagnoses, were known to have no effects on bone metabolism, were matched by sex, age and race with 50 healthy control subjects. Cases had received maintenance warfarin therapy, an antagonist of vitamin K, for more than 1 year. BMD was measured at spine (L_1-L_4) and four femoral sites. No difference was seen between cases and controls in BMD at all measured skeletal sites. Separate analyses for sexes did not show any difference between men and women. Age, sex, smoking, physical

activity, calcium intake and HRT were considered in the analyses. Duration of warfarin use greater than 10 years also was not associated with a significant decrement in BMD.

In the second part of this study, 113 healthy elderly community-dwelling subjects aged over 65 years (35 men and 78 women) were recruited to evaluate associations between plasma concentrations of vitamin K and PIVK-II (protein induced by vitamin K absence) with BMD in patients who were taking warfarin for more than one month. Bone measurements in this part and confounders were similar to the part one and produced similar results; there was no correlation between BMD at all skeletal sites and plasma PIVK-II or vitamin K levels for both sexes, separately or combined together. However, in the first part of this study, cases were not similar to the controls in terms of health condition. Additionally, dietary intakes of other nutrients were not considered. Using medication has limited the generalizability of the findings.

2.2.5.3. Summary

In conclusion, prospective studies showed the protective effect of vitamin K on the risk of osteoporotic fractures (191;192) and a case-control study confirmed these associations (193), although there was no clear association between vitamin K intake and BMD at several skeletal sites (192). Vitamin K antagonists were not also associated with BMD at different skeletal sites (195). Threshold effect of vitamin K with a possible threshold of 109 μg/d in a prospective study (191), together with the positive association between vitamin K supplementation and under-carboxylated osteocalcin (194) may indicate that the recommended amount of 1 µg/kg/d is insufficient to provide a good bone health in the community. However, evidence regarding to the needed amounts of vitamin K for bone health seems to be inadequate to allow an obvious conclusion; Feskanich et al (191) found a threshold level of 109 μg/d (for dietary vitamin K) beyond which no further decrease in fracture risk had happened, others have suggested levels far higher than this level to maximize the decrease of under-carboxylated osteocalcin; for example, Binkley (196) suggested a level of 1000µg/d. It is worth noting that under-carboxylated osteocalcin is shown to be associated with osteoporotic fracture risk (197). It is also of importance to distinguish between pharmacological effects of high dosage of vitamin K with those of usual dietary intakes in conjunction with intestinal produced amounts of vitamin K. Fur-

thermore, there is no evidence to show long-term benefit of such supplementation for fracture rates.

With regard to the effect of vitamin K on bone and hip fracture risk it should be noted that the main sources of vitamin K are green vegetables, which are rich in other nutritional compounds effective on bone metabolism. Interaction between vitamin K and other nutrients is also important such as vitamin D, which was significant in the cohort study cited before (191). In this study, women with low vitamin K(<109 μg/d) but high vitamin D (>8.4 μg/d) intakes had a greater risk of hip fracture (RR = 2.17, 95% CI, 1.19-3.95) than women with low vitamin K and low vitamin D intakes (<4 μg/d). Fracture risk was not reduced in women with high vitamin K (> 109 μg/d) and high vitamin D intakes. No interaction between calcium intake and vitamin K intake on bone was observed. However, other nutritional variables are not considered in the presented studies.

2.2.6. Caffeine

Caffeine is one of several xanthine-derivatives, which is naturally found in coffee beans, tealeaves, kola nuts and cocoa beans. It is an alkaloid compound and its chemical formula is 1,3,7,trimethyl xanthine. Pure caffeine is odourless and has a bitter taste (5;198).

2.2.6.1. Dietary source and intake

Typical dietary sources of caffeine are coffee, tea, cocoa, and cola beverages and also a relatively insignificant amount of caffeine, which may enter the diet through other foods such as coffee-flavoured ice-cream, chocolate bars and other chocolate-flavoured foods. Among these sources, coffee is the most popular beverage in most western countries and supplies over 80% of total caffeine consumption in these countries (198). However, the primary contributor to caffeine intake in the UK is tea (452). Figures from a recent nutritional survey in the elderly population in the UK (59) indicated that more than 95% of the elderly people in the UK drink tea, while only 40% of people in institutions and 60% of people free-living elders drink coffee. The amount of tea drunk by tea consumer was considerably higher than the amount of coffee drunk by coffee drinker (24 cup/week vs. 7cup/week in free-living individuals and 25cup/week vs. 4cup/week in persons living in institutions).

The caffeine content of different related foodstuffs and beverages varies greatly and in coffee and tea it is influenced by brewing time and strength, the particular brand (plant variety) geographic location, climate and cultural practices including fermentation (198).

2.2.6.2. Biological effect of caffeine

Ingested caffeine is rapidly absorbed, metabolised and excreted in the urine as methyl xanthine derivatives. Its metabolic half-life in plasma and most organs is about 3hours and its excretion is primarily renal (198). Its diuretic and stimulating effects are well investigated but will not be explained here. The effects of caffeine on osteoporosis and osteoporotic fractures are of interest in this review. Theoretically, caffeine causes an increase in urinary calcium excretion for 1-3 h after ingestion, but the long-term calciuric effect on 24h-calcium excretion is controversial. Additionally, it may exert a direct effect on bone metabolism via cyclic adenosine monophosphate (CAMP).

2.2.6.3. Caffeine consumption, calcium metabolism and bone health

In respect of the various effects of caffeine on calcium balance, osteoporosis and fracture risk, investigations showed inconsistent results. Several studies reported no association between caffeine intake and bone health (199-203)and numerous have reported a positive correlation between coffee consumption and osteoporotic fractures or bone loss(204-206). In this section epidemiologic and metabolic studies will be presented, separately.

Epidemiologic evidence

Kiel et al (207) conducted a prospective study in a big cohort (3170 men and women aged 50-84) for 12 years. It was found that the consumption of 2.5- 3.0 cup/day of coffee increases the risk of hip fracture significantly in women (RR= 1.69, 95% CI, 1.05-2.74), but for men this relation was not statistically significant (RR=1.42, 95%CI 0.59-3.38). Among women the youngest group (less than 65 y) was at the highest risk. With increasing age the risk decreased significantly. Authors concluded that caffeine intake of more than two cups per day is associated with an increase of 53% in the risk of hip fracture in the subsequent 2 years. However, the number of hip fractures in this study was low (a total of 135 fracture over 12 years), especially in men and the authors failed to consider many confounders, even some important variables such as calcium intake and physical activity.

Another cohort study (205) produced similar results regarding to the risk of hip fracture in women. In this study, 84484 women aged 34-59 years were followed-up for 6 years. Relative risk of hip fracture for women in the top quintile of caffeine consumption in comparison with the lowest quintile was 2.33, (95% CI 1.18-7.38, P = 0.003). However, wrist fracture was not associated with caffeine consumption. Age, alcohol intake, BMI, menopausal status, oestrogen use and calcium intake were considered in this study.

Contrasting to the presented studies, Holbrook et al (152) reported no association between caffeine consumption and hip fracture among 957 men and women (531 women and 426men) aged 50-79 years, who participated in a prospective study, over 14 years of follow-up (RR = 1.1, P was not significant, [>0.05]) (for details of this study see the section 2.2.4).

Similarly, three case-control studies have shown no effect of caffeine on hip fracture risk (161;202;203). Kreiger et al (161) reported a case-control study in 533 women aged 50-84 y (256 cases of hip and wrist fracture and 277 age-matched controls admitted to the same hospital of the cases) and found no association between caffeine consumption and hip or wrist fracture risk. In this study, OR of hip fracture for drinking of 3 or more cup of coffee was 1.18,(95% CI, 0.64- 2.18) and that of wrist fracture was 0.90 (95% CI, 0.55- 1.47). ORs were adjusted for BMI, ovariectomy, oestrogen replacement therapy (HRT) and cigarette smoking.

Likewise, Nieves et al (202) published a case-control study in 329 postmenopausal women aged 50-103 years (161 hip fractured cases and 168 hospitalized controls) and found no association between intake of caffeine and hip fracture risk. Power of study was 75% (to detect an OR of 2 for comparison between lowest and highest quartiles) and response rate was 61% in cases and 56% in controls.

Another case-control study by Tavani et al (203) also found no relation between caffeine consumption and hip fracture risk in women. OR for hip fracture in coffee-drinkers was 1.2(5%CI 0.8-1.7), compared with non-drinkers. No association was found for the number of cups/day, and no for duration of coffee intake with hip fracture risk. Similarly, no statistically significant association was found with decaffeinated coffee, tea, or cola intake.

Results were controlled for age, education, BMI, smoking status, alcohol use, calcium intake, menopausal status, and HRT.

Relationship between bone measurements and caffeine consumption has been also investigated but revealed contrasting results.

Yano et al (206) examined the relationships between diet and BMC of the radius, ulna and os calcis among 1368 men aged 61-81 and 1098 women aged 43-80. They found a negative association between caffeine intake and bone density particularly at the distal forearm (distal of radius and ulna) ($P<0.01$) only in women. No similar associations were found in men. For proximal forearm and Os calcis associations were negative but not significant. Age, height, body weight, physical activity, thiazide and oestrogen use were considered in the analyses.

Barrett et al (204) reported a cross-sectional study in 980 postmenopausal women, aged 50-98 and reported a negative association between caffeine consumption and BMD of the hip and lumbar spine. BMD of femoral neck, total hip and lumbar spine decreased significantly with increasing lifetime cup-years of caffeinated beverages. These associations were independent of age, alcohol drinking, physical activity, smoking, HRT and years since menopause. However such associations were found only in those who did not drink at least one glass of milk every day from the age of 20 to 50y. Milk added to coffee was not considered. Overall nutrient intakes and even calcium intake were not considered in this study.

Cooper et al (208) has also reported a negative association between caffeine consumption and bone density just at femoral shaft (among six skeletal sites: spine, two forearm sites and three femoral sites) and in old women (60 years or over). Surprisingly, they observed a favourable effect of caffeine on BMD at femoral shaft in women under 50, for example, as they reported, each 100mg increase in daily caffeine consumption at age 50 and 40 was associated with an increase in BMD at femoral shaft of 0.4% and 1.0% respectively. In contrast, the same amount of increase in daily caffeine consumption at age 60, 70 and 80 was associated with a reduction in femoral shaft bone mineral of 0.2%, 0.9% and 1.7% respectively. Therefore, they concluded that the effect of caffeine on bone health is related

to the age and the skeletal site. The effect of age in this study is in contrast with the results reported by kiel et al (207). However, they did not find any association between caffeine consumption and bone density at five other skeletal sites. They also reported no relation between calcium intake and caffeine consumption. Response rate in this study was low (60%) and respondents were not similar to non-respondents in respect of health status.

Contrasting to the above-mentioned studies, many other studies have reported no association between bone measurements and caffeine consumption in both sexes. Johansson (200) reported no significant association between calcaneal bone mass, and coffee consumption among 619 men and women aged 70 years, after controlling for height, body weight, smoking habits and physical activity. In this study however, just calcium intake was evaluated from three dairy foods (milk, yoghurt and cheese) and high calcium intake in their population may have affected the results.

Similarly, no relation was found between caffeine and bone density at hip and total body in 138 healthy postmenopausal women aged 55-70y (201). The caffeine intake, measured by gas chromatography of each subject's brewed caffeinated beverages, ranged from 0-1400 mg/d. and categorizing the subjects by their caffeine intake as high, medium or low consumers showed no difference between different levels of caffeine intake.

However, caffeine consumption maybe associated with the rate of bone loss. Harris et al (199) conducted a study in a cohort of 205 healthy, non-smoking postmenopausal women (mean age of 61 years) and correlated 1y changes in BMD of the spine and total body to the caffeine consumption and calcium intakes. Caffeine consumption was found to be negatively related to 1-y bone density variation in women with low calcium intake (< 744 mg/d), but in women with calcium intake near or above the RDA for USA population of 800mg/d no association was found. Years since menopause, BMI and physical activity were taken to account in the analyses. In this study, caffeine consumption was positively associated with dietary calcium intake ($r = 0.27$, $P<0.001$).

Metabolic studies

Five human metabolic studies have also provided conflicting results. Heaney and Recker (209) in their study on 170 premenopausal women with mean age of 29, showed a nega-

tive effect of caffeine consumption on calcium balance via increasing urinary and faecal calcium excretion. A 50% increase in caffeine intake above the group mean intake value, resulted a shift of –6mg/day in calcium balance. The mean caffeine consumption in their population was 350 ±232mg/day and mean calcium intake was 660 ±342mg/day.

Another study by Hasling et al (210) in 85 postmenopausal women with spinal osteoporotic fracture yielded a negative effect of caffeine consumption on calcium balance ($r = -0.21$, $P = 0.05$), but mineralization rate was not related to the caffeine consumption and other dietary constituents. They showed that a caffeine intake in excess of 1000 ml could induce an extra calcium loss of 1.6mmol calcium/day (6.4mg/d). However, since all subjects in this study were patients with osteoporotic fracture, the results may not be applicable to the general population.

Messey et al (211) have studied the effect of caffeine abstinence on calcium and bone metabolism in women, habitually consuming caffeine. 25 women of age 39-76 years were participated and based on their calcium intake were divided into two groups: the group of low calcium intake (<600 mg/day) or moderate calcium intake (660-1357mg/day). It was found that after two weeks of caffeine abstinence, fasting serum ultrafiltrable calcium increased and serum bone alkaline phsphatase isoenzyme levels decreased only in women consuming less than 600mg calcium daily. Other parameters such as magnesium, phosphate and nitrogen did not differ across two groups of low or moderate calcium intake and in caffeine consumption or caffeine abstinence periods. Fasting total serum calcium, hydroxyproline/creatinine and calcium/ creatinine excretion did not vary between caffeine consumption and caffeine abstinence periods or between two calcium groups. The authors concluded that caffeine consumption of more than 175 mg/day in those women with low calcium intake (less than 600 mg/day) may negatively affect calcium and bone metabolism. However, the mean age differs between the two groups (64.6 for low calcium group and 49.1 for high calcium group) and other nutrients were not considered. Furthermore, vitamin D nutritional state was not controlled for, in this study. Vitamin D is determinative for adaptation mechanism in calcium metabolism.

Barger-lux et al (212) conducted a study on 16 healthy premenopausal women with mean age of 29.5 years. They found that a moderate dosage of caffeine (400mg/day) lowered

calcium balance, but the effect was not statistically significant. No effect of caffeine on faecal and urinary calcium was also observed. High calcium intake in their subjects (14.1-39.7 mmol/d median = 21.5mmol/d) may have affected the findings.

Massey et al (213) in their study on 12 healthy females, aged 22-30 (mean 24.5 years), reported a weak and non-significant dose-dependent diuretic effect of caffeine and an increase in total three hour urinary excretion of calcium, magnesium and sodium. Urinary excretion of calcium was related to a combined effect of increased concentration and urinary volume. The urinary concentration and total excretion of calcium at each of three hourly sample points was significantly related to the dose of caffeine. Their method of adding caffeine to a decaffeinated coffee and tea is appreciable for ensuring about the exact amount of ingested caffeine. However, small sample size, lack of data about calcium excretion after 3rd hour of caffeine drinking, lack of information about calcium intake and missing many confounders are the weaknesses of the study.

2.2.6.3. Summary

In conclusion, studies of caffeine consumption and bone health have produced inconsistent results. In this regard, some considerations may be of importance; first, as noticed before, the amount of caffeine in coffee and other dietary sources of caffeine varies greatly according to the brand, brewing time and strength, but in most studies it was not considered, except in three studies in which the exact caffeine concentration was determined (201;210;213). Second, most of the presented studies did not account for many confounders as well as nutrients that may affect calcium metabolism and bone health, especially that of vitamin D, which is a major determinant of calcium absorption and adaptive mechanism. Third, the effect of other components, which may accompany the caffeine in beverages, might be of importance and vary among different brands and beverages and their effects on bone are not considered, such as polyphenols and other alkaloids. Fourth, caffeine may be associated with other behaviours that are themselves risk factors for bone health and fracture risk, such as alcoholism and cigarette smoking (208;211).

Metabolic studies have shown that the effect of caffeine is depends upon calcium nutritional state. Subjects with higher calcium intake are less likely to be inversely affected by caffeine intake (199;211). On the other hand, net calcium absorption and urinary excretion

is mainly determined by dietary constituent of calcium (210) these data suggest that caffeine mainly affects calcium metabolism, an effect which may be temporarily and last less than few hours and be compensated by adaptive mechanism. Both adaptive mechanism and the effect of variation in calcium metabolism on bone are under influence of other factors such as age, nutritional state and especially vitamin D and hormonal state. Therefore, age, sex and menopausal state in women are major confounders and the effect of caffeine should be considered in the context of the entire diet, in which variations in different nutrients and non-nutrient components are taken into account.

It appears that the effect of caffeine on bone is site-specific and to some extent may explain discrepancies between the results. This effect was shown in different studies (205;208) and is similar to the results of other studies of relations between other factors and bone health, discussed before (for example; those of protein, vitamin D and calcium). This may suggest that local factors are in operation. Differences between skeletal sites in terms of trabecular or compact proportions, local forces, local blood supply may provide explanation.

2.2.7. Alcohol

Ethyl alcohol (ethanol, C_2H_5OH) is the principal alcohol in nature of nutritional significance and methanol is a cheap alternative to alcohol with a high risk of intoxication, retinal damage and permanent blindness. Ethanol has been produced by most civilisations by fermenting carbohydrate, particularly sugars (5).

2.2.7.1. Alcohol absorption and metabolism

Alcohol is absorbed mainly in the upper gastrointestinal tract. Its absorption can be delayed if alcohol is taken slowly throughout the course of a meal or when preceded by a drink of cream or oil. Some of the alcohol in the stomach is immediately metabolized by alcohol dehydrogenase enzyme, which is found in the gastric mucosa. This first-pass metabolism of alcohol is reduced in chronic drinking because the enzyme level falls as gastritis develops with heavy drinking. Once absorbed, the alcohol spreads rapidly throughout the body water space (58).

In a healthy person alcohol is cleared from the body by the liver at a constant rate of 15 mg/100ml blood per hour. This rate of metabolism differs markedly from person to person and is influenced by drugs and hepatic adaptation (58).

2.2.7.2. Biological effects of alcohol on bone health

Alcohol has both direct and indirect effects on bone metabolism. The effects of alcohol on liver, nervous system and nutritional status are indirectly related to the bone metabolism and osteoporotic fractures. Chronic alcohol intake produces liver damages, which may progress from fatty infiltration to hepatitis, fibrosis and cirrhosis (5;58). These damages are mainly mediated by free radicals, which are produced by cells during ethanol metabolism. Similar effects can be seen in pancreas in alcoholics, which can lead to chronic relapsing pancreatitis (214). These pancreatic and hepatic changes are the main cause of malnutrition in chronic alcoholism. In addition, ethanol exerts a direct toxic effect on calcium absorption and bone metabolism (214).

The effects of alcohol on nervous system and muscle function are also of importance; alcohol directly affects the nervous system both central and peripheral. In the central nervous system, as blood level of alcohol rise, individuals feel that they have greater ability, when in fact their motor and intellectual performance is deteriorating. In the peripheral nervous system alcohol leads to sensory loss and motor dysfunction, which in turn can weaken the tendon reflexes (58). Additionally, heavy ethanol drinking leads to muscle weakness due to a direct effect of alcohol on striated muscle tissues (5;214).These neuromuscular effects of alcohol may be important in increasing the risk of fall in alcoholics (5;19;58).

2.2.7.3. Alcohol intake and bone health

Published studies on alcohol and bone health have produced inconsistent results. Holbrook et al (215) published a prospective study for a follow-up period of four years on a cohort of 449 subjects (182 men and 267 women aged ≥ 45 at baseline) investigated the relationship between bone density and alcohol consumption. Bone density was measured at four skeletal sites (radial shaft, ultra distal wrist, femoral neck and lumbar spine). Alcohol consumption was measured at baseline by two interviews: one ascertained weekly al-

cohol consumption from all alcoholic drinks and the other measured alcohol intake in the previous 24-h. Bone mineral density in all skeletal sites increased with increasing alcohol intakes in men and women, but the results were significant for femoral neck in men ($P<0.01$) and spinal site in women ($P<0.01$). BMI, age, physical activity, smoking and HRT in women were taken to consideration. According to this study, the effect of alcohol on bone may vary by sex and differ by the skeletal site. However, because of the sampling method of this study, the study sample was not similar to the general population.

A cross-sectional study also reported positive association between alcohol intake and bone mass in women (216). Usual dietary intake (over the previous 12 mo) of 62 healthy women aged 45-55 years was assessed by using a food-frequency questionnaire and were analyzed in relation to BMD at femoral, radial and spinal sites. Age, weight, height, age of menarche, menstrual status, parity, smoking habits, present and past physical activity levels, socioeconomic status, caffeine intake were also taken to consideration. Femoral and spinal BMD were not related to alcohol intake while, total radial BMD was significantly associated with alcohol intake ($r = 0.35$, $P<0.005$). Trabecular and cortical forearm BMD were also related to alcohol intake ($r = 0.32$ and $r = 0.18$, $P<0.01$).

Similarly, Felson et al (217) reported positive association between alcohol intake and BMD at femoral, spinal and radial sites in 1154 participants in Framingham Heart Study Cohort. On average, women with alcohol intake more than 207 ml/week had 7.7% higher bone density at all measured skeletal sites. Among men drinking of 414 ml/week of alcohol was associated with an increase in BMD of on average of 3.9%. Age, weight, height, smoking, and, in women, age at menopause and years of oestrogen use were taken to consideration.

Contrasting to these results, several case-control studies found negative association between alcohol intake and bone mass. One early study in this regard published by Nilson et al (218), who compared the BMC of forearm bones in two groups of cases with a group of controls. Controls were recruited from an outpatient orthopedic clinic (56 men, mean age 45-y) and two groups of cases were recruited from the same hospital: one group (58 men, mean age 45-y) consisted of the patients admitted voluntarily to the department of alcoholism and the other group consisted of 35 male patients (mean age of 52-y) admitted or

seen as outpatients in the department of orthopedic surgery. The diagnosis of alcoholism in the latter group was based on criteria such as a history of delirium tremens. BMC of distal and proximal forearm (measured by gamma-ray absorptiometry) was lower in alcoholics when compared to those of controls ($P<0.05$) (for example; the means of BMC for distal forearm measurements were 459 mg/cm^2 in controls, 408 in alcoholic department patients and 375 in orthopedic department cases). Nutritional variables other than alcohol were not considered and many confounders were neglected. Additionally, the amount of alcohol consumption and its duration as the exposure of interest were not properly evaluated. Furthermore, diagnostic criteria of alcoholism in one group of cases (orthopaedic department cases) were not precisely defined.

Another case-control study (219) was performed on 30 men admitted at an alcoholic research clinic, who had been consuming excessive amount of alcohol for an average of 18 years (7- 40 years), and 76 non-patient subjects as controls. Physical activity, the period of drinking and eating pattern were taken to consideration. BMC at six skeletal sites (femoral neck and shaft, shaft and distal of radius and ulna, head of humerus and calcaneous) was compared between cases and controls. At all skeletal sites, adjusted BMC (for age and body surface) in alcoholics was lower than in non-patient subjects by a mean difference of –3.3%. This difference for femoral neck was –4.7% (P <0.001).

Studies that relate alcohol consumption to osteoporotic fracture rate are relatively consistent and show a deleterious effect of alcohol on the risk of osteoporotic fractures. Felson et al (220) published a prospective study on a cohort of 5,209 aged 28-62 years at baseline for a follow-up period of about 35 years. Heavy alcohol consumption (≥207 ml/ week) was associated with a higher risk of hip fracture, but the effect varied among men and women. Age-adjusted RR of hip fracture among women in the highest third (≥207 ml/ week) was 1.54 (95% CI 0.92-2.58) compared with the lowest third (<29.5 ml/ week) (P = 0.05). Equivalent RR for men was 1.26 (95% CI 0.62-2.55). The effect of alcohol consumption on bone was not similar in different ages. RR for men and women aged less than 60 and with the highest third of alcohol intakes were 9.90 (95% CI 1.80-53.35 P <0.01) and 2.96 (95% CI 1.24-7.1) respectively, when compared with the lowest third. These RRs decreased across the subsequent age groups (10 years intervals). For the entire

cohort, current alcohol consumption was associated with a modest, statistically significant increased risk of fracture, with a relative risk of 1.28 per 207ml/week alcohol consumption. In those younger than age 65, the RR associated with heavy alcohol consumption was higher (Odds ratio = 1.40 95% CI 1.07-1.84) than in those aged 65 years and over (OR = 1.17 95% CI 0.90-1.53).

Another prospective study (205) in a cohort of 84,484 women, aged 34-59 years, for a follow-up period of six years have investigated the association between alcohol consumption and hip fracture risk. A significant increase in the risk of hip and forearm fractures in women was found with a moderate alcohol intake (5-24g/d). Adjusted RR of hip fracture for women who consumed 25 g/d alcohol or more, in comparison with nondrinkers was 2.33 (95% CI 1.18-4.57, P =0.04). RR was adjusted for BMI, menopausal status, HRT and calcium intake. Similar RR for forearm fracture was 1.38 (95% CI 1.09-1.74).

Case-control studies have reported similar results. Clark et al (221) published a case-control study in a total of 295 men and women aged >45 years. Alcohol intake was associated with increased risk of hip fracture in men and women with an OR of 1.73 (95% CI 1.04-2.90 $P<$ 0.05) for drinkers in comparison with non-drinkers ($P\leq$ 0.03). Smoking, physical activity, the number of pregnancies and breastfeeding in women were taken into account. Cases were different from controls in terms of calcium intakes (490 ± 245 in cases vs.576 mg/d ± 297 in controls, $P \leq 0.007$).

Another case-control study by Cooper et al (159), cited before (see section 2.2.4), has shown similar results. In this study, moderate and heavy drinking was associated with a RR of 7.5 (95% CI 3.3 –16.8) and light drinking was associated with RR of 1.3 (95% CI 1.0-1.8) in comparison with subjects who have never drunk or have drunk occasionally. The result of this study is in keeping with another study by Lau et al (155), cited earlier (see section 2.2.4), which showed that the risk of hip fracture for daily alcohol consumer was 3.9 (95% CI 2.3-6.7) compared with no consumer ones.

Another case-control study by Nilsson BE (222) on 70 hip fractured male patients under age of 70, and 125 age-matched controls showed a significant high frequency of chronic alcoholism in cases versus controls (19% vs. 2% $P<0.001$).

2.2.7.4. Summary

There appears to be sufficient evidence to allow the conclusion that alcohol intake is harmful for bone health. Despite evidence indicating a possible salutary effect of social alcohol drinking on BMD at some skeletal sites such as femur and spine (215;217), or radial bones (216), there is no evidence to show any protective role of alcohol for fracture risk. Rather, almost all studies (both prospective and case-control studies) have reported increasing of fracture rate with regular consumption of even slight amounts of alcohol beverages (159). The positive role of alcohol in bone density measures seems to be of no significant benefit and may be attenuated by other harmful effects, such as effects on sensory and motor nervous system leading to increasing the risk of fall. Any change in bone mineral status would be of benefit only if can result in decreasing the rate of osteoporotic fractures. In the Framingham Heart Study (220), although alcohol intake was associated with increasing BMD at several skeletal sites, it increased the risk of hip fracture. However, the positive association between alcohol intake and bone mineral status is still a matter of controversy and has been refused by several studies (218;219).

Positive association between bone mineral and alcohol intake may be because of augmentative effect of alcohol on oestrogen (217). In this regard, disentangle between the effect of alcohol from those of other compounds in the alcoholic beverages, such as; flavonoids, antioxidants and hydroxystilbenes is difficult (223).

On the other hand, the harmful effect of alcohol maybe due to different lifestyle and nutritional habits in alcoholics in comparison with non-alcoholic persons. Direct toxic effects of free radicals due to alcohol metabolism and high prevalence of malnutrition in alcoholics in comparison to nondrinkers may be other reasons (214). In this regard, distinguish between healthy subjects, who are interested in social drinking and are considered as light drinker without any health or nutritional problem, with alcoholic persons, who are heavy drinker and are more likely to suffer from other health problems or nutritional deficiencies may be of importance. However, most studies in this regard failed to assess variation in food and nutrient intakes in relation to alcohol, which are clearly important for bone health. It appears that drinking exerts slightly different effects in men and women. These differences were also evident for other nutritional and non-nutritional variables regarding

to bone health and osteoporotic fractures. Variation between different skeletal sites in response to alcohol intake is also similar to that explained earlier for other bone affecting variables.

2.3. Non-nutritional variables and bone health

There are many variables other than nutritional ones, which are important in the pathophysiology of osteoporosis. Clear understanding of their impact on bone health is essential for planning of any study on nutrition and bone health.

2.3.1. Weight

Theoretically, body weight is an important determinant of skeletal integrity and bone health:

1. Body weight determines the mechanical loading strain on bone and skeleton, which is important for bone remodelling and bone strength (see section 2.1.2.2).
2. The skeleton in heavy persons is more supported by the protective effects of soft-tissue padding against opposing forces in trauma (48).
3. Greater proportion of adipose tissue in obese subjects may increase serum oestrogen due to greater conversion of adrenal androgens to oestrogen, which is known as a beneficial factor for bone health (17;224).
4. Low body weight maybe a marker of poor nutritional status, which is a risk factor for bone health.
5. Weight loss increases the risk of fall in the elderly (225) and may decline the ability of diminishing the energy of a fall.

Negative association between weight and vitamin D state has also been noted and will be discussed here.

2.3.1.1. Weight, weight change and bone health

Association between, body weight, weight change and the risk osteoporotic fractures have been investigated in many studies.

Cumming et al (226) in a prospective study on 9516 women aged 65 or more and with no history of hip fracture, had evaluated the association between weight change and hip fracture risk over a period of 4.1 years. It was found that a 20% weight gain between age 25

years and old age was associated with a 40% reduction in the risk of hip fracture. Current body weight and BMI did not remain significantly associated with the risk of hip fracture after adjustment for weight gain. Results were adjusted for physical activity, smoking, alcohol use, menopausal status, medication and weight of the age of 25. Inevitable recall bias for remembering the weight of the age of 25 was the main limitation of this study.

Another study by Ensrud et al (225) was carried out on 6754 ambulatory women aged over 65, for a follow-up period of 19.5 month on average. Subjects were enrolled in a cohort study on osteoporotic fractures for a mean period of 5.7 years and their weights were measured at the baseline and the end of this period, then they were followed-up for incident fractures during 19.5 months for this study. On average, subjects lost 1.5% of their weight (SD = 7.7%) during the first period (mean 5.7 years). Interestingly, women who tried to lose weight gained weight since baseline (mean 0.83%), whereas those not trying to lose weight, on average, lost weight (mean –2.47%). There were also different relative risks of osteoporotic fractures for weight loss in these two groups; RR for each 10% decrease in weight among the women who were not trying to lose weight was 1.8 (95% CI, 1.3-2.6). Equivalent RR among the women trying to lose weight was 0.85 (95% CI 0.37-1.94). In this study, each 10 % decrease in weight increased the risk of frailty fractures by 86%. Age adjusted RR per 10% decrease in weight for hip fracture was 2.1 (95% CI 1.4-3.0). Neither current weight nor standard BMI was statistically significantly related to the risk of fractures after adjustment for percentage of weight change, but the magnitude of the effect of weight change was not similar among subjects weighted lower than 65kg and those with higher weight. In the other words each 10 % decrease in weight among women with a current weight ≤65 kg was associated with a significant 2-fold increase in risk of frailty fractures, while the same weight loss in women with a current weight greater than 65 kg was associated with a non-significant 1.3-fold increase in the risk. Weight gain in this population was associated with a 41% decrease in the risk of osteoporotic fractures; RR for women who gained weight more than 2.9% compared with those with stable weight was 0.59 (95% CI 0.27-1.29). In this study however, nutritional status and nutrient intakes were not considered, which maybe different between subjects who lost weight and those who did not.

Another prospective study for a period of 18 month was performed by Salamone et al (227) on 236 healthy premenopausal women aged 44-50 years. They carried out a clinical intervention trial in which subjects were randomised into two groups: an intervention group (n=115) who involved in a lifestyle intervention, designed to lower fat intake, increase physical activity (increasing energy expenditure) and a modest weight loss, and a group of controls (n = 121). A calcium supplement of 1200 mg/d was recommended for intervention group. The two groups were almost similar at baseline in terms of age, weight, BMI and dietary intake. Intervention group lost their weight by 3.2±4.7 kg (4.5± 6.4% of body weight) over the 18-mon period whereas control subjects gained weight by 0.42±3.6kg (0.69±5.2% of body weight) (P <0.001). Annualized rate of bone loss at the spine and hip were almost twice as high in the intervention group as in the control group (P = 0.015). Women in the top quartile for weight loss (8.0% or more) had >3 times the bone loss of all other women even after adjustment for baseline BMD. However, differences between intervention and control group in terms of dietary intakes, physical activity and calcium supplementation may have affected the results.

Association between body weight, weight change and bone health was also investigated in a case-control study performed by Cuming et al (21). This study was performed in 416 subjects in Australia (209 cases and 207 controls) who were over 65 years old. Adjusted odds ratio (for age and sex) for subjects with highest BMI (27 in males and 26 in females) in comparison with the lowest ones (21 in males and 18 in females) was 0.3 (95% CI 0.1-0.8). Similar ORs for BMI at the age of 20 and 50 were 1.6 (95% CI 0.7-3.7) and 1.1 (95% CI 0.4-2.9), respectively. Compared with people who maintained or put on weight after the age of 50, the odds ratio for people who lost weight was 1.9 (95% CI 1.1-3.3). The equivalent OR for weight loss after age of 20 was 3.4 (95% CI 1.8-6.4). Recall bias and imprecise measurement of weight and BMI of previous ages may be the main and inevitable limitations of this study. The results of this study were consistent with cohort studies, except the significant effect of current weight and BMI, which also was reported by a cross-sectional study, cited before (206) (see section 2.2.6.3). In this study, current weight was positively related to the BMC at all measured skeletal sites (proximal and distal of forearm bones and Os calcis) in men and women (P<0.001).

Vitamin D status is an important variable for bone health. Therefore, the effect of weight and BMI on vitamin D status is of particular importance. In this regard, Jacques et al (74) in their study, cited before (section 2.2.3.), found that the plasma concentration of 25(OH)VitD in men and women was 1.1 and 0.8 nmol/L lower for each unit increase in BMI, respectively.

Interaction between weight and vitamin D status was also noticed in a study by Compston et al (228) on 24 grossly obese subjects. Their population were comprised of 24 grossly obese patients aged 22-49 y (mean 34.5-y, two male and 22 female with a weight range of 96-155kg) and a control group of 20 healthy subjects aged 19-52y (5 male and 15 female [their weight is not available]). They showed that the plasma concentrations of 25(OH)VitD were significantly lower in the obese group than in age-matched, healthy control group (25.1 vs.32.5 nmol/L $P<0.05$) with a prevalence of 25% of hypovitaminosis D in the patient group. The mean trabecular bone volume in the patients was also lower than controls (25.6% vs. 28.8%). Small sample size, lack of data of dietary intake of vitamin D and other nutrients among subjects as well as missing other confounders such as physical activity and outdoors spending time are among the main limitations of this study.

BMI is an important variable influencing the protective effect of vitamin D supplementation among women against osteoporosis. This has been shown in a case-control study by Ranstam et al (229) in 1634 hip fractured females aged 50 y or older and 3532 age-matched controls from Sheffield. In this study, among women with a BMI less than 20 kg/m^2, use of vitamin D was associated with reduction in hip fracture risk of 55% (RR = 0.45 95% CI, 0.24-0.84 $P = 0.01$), whereas in women with higher BMIs the protective effect of vitamin D was not significant (for example; RR for women with BMI 20-24 was 0.80, 95% CI, 0.49-1.31). However, this study was conducted in several European countries and the results maybe affected by cultural, environmental and genetical variables. Furthermore, using vitamin D was investigated in this study, while the vitamin D status of the population was not clear.

2.3.1.2. Summary

According to the current evidence, weight change is more related to bone health than the current weight or BMI in both sexes, but magnitude of this effect is under influence of

current weight (225). In almost all studies weight gain was associated with bone gain or lower chance of frailty fractures and conversely, weight loss was associated with bone loss or greater chance of frailty fractures even in circumstances in which weight loss is induced by controlled interventional trials and by increased physical activity, which is known to favourite bone health (227). In this regard, dietary changes leading to weight change are of particular importance and may provide an explanation for the effect of weight loss on bone. This issue has been neglected in almost all studies but may be of benefit to reduce the negative effect of weight loss on bone health, especially for those who must lose their weight because of some medical or health considerations.

Another issue in this regard is the effect of duration of weight change, which has not been considered in the presented studies. The time period, in which weight changes happened might show the intensity of restrictions on dietary intakes or other interventions, which may be directly related to the bone metabolism

The effect of current weight or BMI in the presented studies was a matter of controversy; some studies have shown no link between current body weight and bone health (225;226) while, some others have reported positive associations (21;206;230).

The effect of weight loss is also influenced by the intention for losing weight. Voluntary weight loss is less harmful than involuntary weight loss (225).

Negative associations between BMI and body weight with serum 25(OH) VitD concentration, which is a marker of vitamin D status, have been reported in several studies (61;74;147;228;229). This effect however, may be due to other factors such as low physical activity and thus, low sunshine score or low density of vitamin D in diets of obese subjects or physiological differences between heavy and light persons, which needs more clarification (228). This effect seems to be in contrary to the effect of weight on bone mass and the risk of osteoporotic fracture. As stated before (see section 2.2.3.2), seasonal variation of vitamin D is inversely related to the body weight and BMI (61;147). It means that subjects with greater BMI are less likely to suffer from hypovitaminosis D during wintertime, which is obviously important for bone health in the elderly. This may be attributed to a larger pool size of vitamin D in fat persons due to greater fat mass.

2.3.2. Cigarette smoking

Cigarette smoking is frequently implicated as a risk factor for bone health and hip fracture. Theoretically, several mechanisms might explain the increased risk of hip fracture among smokers:

1. Smoking increases the concentrations of free radicals, which have been suggested to be involved in bone resorption and cell intoxication (231).
2. Smokers have lower fractional absorption of calcium due to the effect of smoking on vitamin D metabolism (232).
3. Smoking is associated with low body weight, which may increase the risk of hip fracture (233;234).
4. Toxic compounds including tars and nicotine and also heavy metals such as cadmium, hydroxyquinones, thyocyante and nitrosamines may directly affect bone metabolism in a negative way (232).
5. Smokers are less active (235), are more likely to use alcohol and caffeine (234) and more likely to have unhealthy eating patterns(236;237).

2.3.2.1. Smoking and bone health

Studies of smoking and bone health have produced inconsistent results. Cornuz et al (233) investigated the influence of smoking on the risk of hip fracture in a cohort of 116,229 females aged 34-59 years at baseline for a follow-up period of 12 years. Smoking was associated with the hip fracture risk in a dose-dependant manner. After adjustment for postmenopausal HRT, menopausal status, physical activity and intakes of alcohol, caffeine and calcium, RR of hip fracture was 1.2 (95% CI, 0.9-1.6) among current smokers and 1.4 (95% CI 0.9-2.1) among those who smoked 25 or more cigarettes per day. Risk did not increase by further increasing cigarette consumption, introducing a threshold effect. Former smokers were not at a greater risk of hip fracture than non-smokers were. Quitting smoking was associated with reduced risk of hip fracture only after 10 years (adjusted RR = 0.7, 95% CI, 0.5-0.9). Their risk was comparable with RR of never smokers (RR = 0.8, 95% CI, 0.6-1.1). RR reduced slightly by increasing BMI, suggesting that the effect of smoking on hip fracture risk may be partially because of low body weight in smokers. When analyses were limited to postmenopausal women results did not change.

Effects of smoking on bone mass has also been investigated in both prospective and crass-sectional studies. Hollnbach at al (234) published a prospective study in a cohort of 504 men and 754 women (aged over 60 at the baseline) for a period of 6 years. Bone mineral density was measured at the spine, hip, and radius (shaft and ultradistal). The mean adjusted BMD at hip in those who were smoker at baseline was significantly lower than in non-smokers ($P<0.05$), but for other skeletal sites, BMD did not differ significantly by smoking status in both men and women. Change in smoking status from baseline was associated with changing of BMD at hip in a dose-dependent fashion in both sexes. Smokers were leaner and tended to use alcohol more than those who did not smoke.

Kiel et al (238) from a cross-sectional analysis on data of 1164 participants in the Framingham cohort, reported that neither current smoking nor recent smoking (past ten years) resulted in a statistically significantly lower BMD at the hip, spine and radius in women who had not taken oestrogen. Among women who had taken oestrogen, BMD at most sites was lower in current or recent smokers than in non-smokers. In men, a consistently lower BMD at all skeletal sites was observed for smokers regardless of when in their life they smoked (4-15% lower). Ex-smoker men who had quit less than10 years ago had lower BMD than men who had quit 10 years ago or more.

Another cross-sectional analysis by Egger et al (235) was performed on a cohort of 410 subjects (224 men and 186 women) aged 61-73 years. BMD at lumbar spine was lower in current smokers by 7.3% (95%CI, 0.4-14.2) and 7.7% (95%CI, 0.3-15.6) in males and females, respectively, in comparison with nonsmokers. Age, weight, height, calcium intake, alcohol consumption, physical activity and menopausal age for women were taken to consideration. The difference at the lumbar spine was statistically significant for men ($P<0.05$) but not for women. Differences in bone mineral density at the femoral neck were smaller and not statistically significant among men and women. For each decade of smoking, BMD at the lumbar spine decreased by 0.015 g/cm^2 in men and women similarly, with slightly different confidence interval values. At the femoral neck the reduction in BMD for each decade of smoking was 0.011 g/cm^2 in men and 0.004 g/cm^2 in women. Therefore, this study showed a stronger negative effect of smoking in men than in women and also a greater effect on lumbar spine than on femoral neck and a greater difference

between men and women at the femoral neck. There was no association between the number of cigarettes smoked per year and BMD at either site among men and women who were ever smoker (evaluation of number of cigarettes smoked was based on maximum daily cigarette consumption reported by the subjects). Physical activity in current smokers was lower than never or ex-smokers in both sexes. However, the method of dietary assessment is not clearly defined and the difference in physical activity maybe resulted in different sun exposure and in turn, different vitamin D status in smokers and non-smokers. Furthermore, dietary habits and nutrients intakes, which are suggested to be different in smokers and non-smokers, are not accurately considered. Additionally, Low response rate among subjects may have led to bias (65% in women and 75% in men).

In summary, although adverse effect of smoking on bone density and the risk of fracture was evident in almost all studies, the magnitude of the effect was the matter of controversy. Bone mass variation by smoking varies by site of the skeleton, with more pronounced effect on lumbar spine and hip (235) in comparison to other skeletal sites (234).

Gender difference was also demonstrated in the presented studies, with stronger effects in men than in women (235;238). The number of cigarettes smoked was determinant in a dose-dependent fashion in two prospective (233;234) but not in a cross-sectional study (235), with a threshold effect (233) and demolishing the adverse effects after about ten years of quitting (233;238). A possible modification effect of hormone therapy on the effect of smoking on bone was also reported and may indicate a metabolic role for smoking or interactions with female sexual hormones (238).

2.3.2.2. Metabolic effects of smoking

The effect of smoking on bone health is partly due to the influences on the metabolism of some nutrients as well as interactions with hormones that are important in the metabolism of bone tissues.

Association between smoking habits and vitamin D status and calcium metabolism was investigated by Brot and colleagues (232). They conducted a cross-sectional study in 510 healthy perimenopausal women aged 45-58 years and 3-24 months after last menstrual bleeding without HRT. The serum levels of 25(OH)VitD, $1,25(OH)_2$VitD and PTH were

significantly lower in smokers compared with non-smokers (9% for vitamin D and 22% for PTH) (P = 0.02), but as expected, there was no difference in serum ionized calcium concentrations between smokers and non-smokers. Hypovitaminosis D, defined as a serum level of 25(OH)VitD below 15ng/ml, was seen among 20.9% of the smokers versus 13.7% of the non-smokers, while Dietary intakes of vitamin D were similar between the two groups. Smokers had lower calcium intake. Age-adjusted BMD at all skeletal sites was lower in smokers than in non-smokers. Smokers were leaner than non-smokers (P = 0.01) and were less active (P = 0.08). This study found a poorer vitamin D nutritional state and lower bone mineral status in smokers in comparison with non-smokers despite similar vitamin D intakes.

Interactions between smoking and hormones are also of importance. Hoidrup et al (239) published a study on modification effect of smoking on HRT in a cohort of 6,159 postmenopausal women for a follow-up period of about 17 years. Considering the menopausal age, HRT, physical activity, alcohol intake, age, BMI and educational level, the risk of hip fracture was significantly associated with cigarette smoking, and physical activity. HRT was associated with a lower hip fracture risk in current and former smokers but not in never smokers. In HRT users in comparison to non-user ones, RR for hip fracture among current smokers was 0.61(95% CI, 0.38-0.99) and for former smoker was 0.55 (95% CI, 0.22-1.37). Equivalent relative risk for never smokers was 1.1(95% CI, 0.60-2.03). Thus, only women who were smoker seem to achieve a protective effect from hormone replacement therapy. Duration, dosage and the onset age of therapy were not considered. Furthermore, their evaluation of HRT and physical activity was performed only at baseline and the possibility of change in these variables was not considered. Finally, nutritional intakes and many other confounding factors were not evaluated.

A further study by Daniel et al (240) has investigated the effect of smoking on steroid hormones and bone mineral density in *young women*. They conducted a case-control study on 52 women (25 smokers and 27 non-smokers) aged 20-35 years. Smokers were tobacco consumers for more than five years (5-21 years) and the mean number of cigarettes smoked per day was 16.9±6.3 (range 8-30). Smokers and non-smokers were not significantly different in BMD at any skeletal site after controlling for age, BMI, physical

activity and skin thickness. The mean serum level of sex hormone-binding globulin (SHBG) in smokers was higher than in non-smokers ($P<0.01$). In contrast, the serum level of free estradiol was lower in smokers than in non-smokers. The authors concluded that moderate smoking has no effect on bone mineral density at any skeletal site in young and premenopausal women. However, the effect of smoking on the serum levels of estradiol and SHBG may be important for bone loss in the elderly as these hormones are important for bone metabolism.

The effects of smoking on bone metabolism are thought to be partially mediated by free radicals and the influence of these radicals might be prevented by dietary intakes of anti-oxidant vitamins. Melhus and collaborates (231) has examined whether the dietary intake of antioxidant vitamins can modify the increased risk of hip fracture associated with smoking. They conducted a case-control study nested within a cohort study. Cases were 247 hip fractured women during 2-46 months of follow-up period (including 44 current smokers and 42 former smokers). Each case was matched to four controls by age and county of residence selected from the cohort (n = 873 including 93 current smokers and 127 former smokers). In this study, among current smokers those with low intakes of vitamin E or C, OR of hip fracture in comparison with never smokers was 3.0 (95%CI, 1.6-5.4). In contrast, current smokers with high intakes of vitamin E or C had ORs of 1.1 (95%CI, 0.5-2.4) and 1.4 (95%CI, 0.7-3.0), respectively, when compared with never smokers. The number of daily cigarette smoked was also associated with hip fracture risk. Current smokers with high cigarette consumption (10 pack-years or more, which means smoking of one pack of 20 cigarettes per day for 10 years or more) had a multivariate OR for hip fracture of 4.3 (95% CI 2.1-8.7) compared with never smokers. Corresponding OR for consumption of less than 10 pack-years was 1.6 (95% CI 0.7-4.1).

2.3.2.3. Summary

In conclusion, current evidence about the effects of smoking on bone health is not consistent and studies with different methods have shown various results. Two cross-sectional studies showed a non-dose response negative effect of smoking on bone density, particularly at lumbar spine (235;238). This negative effect was more pronounced in men than in women (235;238), and was associated with duration of smoking not with the number of

cigarettes smoked per day. Prospective studies have shown different results; they showed a dose-response relationship between smoking and hip fracture risk or bone loss at the femoral neck (233;234), with a threshold effect (233). The effect of smoking may last for a period of ten years (233;238).

Metabolic effects of smoking are also important. Studies on interaction between sex hormones and smoking have shown different results; one (238) has reported lower BMD of different skeletal sites in smokers than in non-smokers among subjects who were using HRT. These differences between smokers and non-smokers were not shown in subjects who did not use HRT. The other (239) has reported a beneficial effect of HRT only in smokers but not in non-smokers. This data suggest that smoking may act as an effect modifier for sex hormones in females. However, this premise has not been confirmed by another study on young women (240) and for men it remains to be discovered.

However, with regard to the evaluation of smoking status and smoking habits all cited studies failed to obtain information about the ways of smoking, including; depth of inhalation, frequency of puff drawing and filter tips, which are important in exposure measurements. Therefore, daily number of smoked cigarettes may not reflect accurately the degree of exposure. Also they did not consider the differences in brand of cigarettes and some aromatic compounds, which are frequently added to the tobacco products and differ greatly in various cigarettes. These synthetic or natural compounds may lead to different metabolic effects. Furthermore, there is no clear evidence to show the contribution of nicotine or other compounds to adverse effects of smoking. In the other words, it is not clear whether the harmful effect of smoking is related to the nicotine content of tobacco or other compounds, which may be avoidable by using special filter tips.

2.3.3. Physical activity

Physical activity is frequently referred to as an effective factor in the maintenance of bone mass. Theoretically, physical activity can influence the bone remodelling process by imposing an additional demand for skeletal strength. Furthermore, active subjects are more likely to have a good health condition and higher appetite and thus higher nutrient intakes (241). However, the results of various studies are not conclusive.

Exercise intervention has been widely used for prevention of osteoporosis and improvement of bone density. In this regard, Smith et al (242) investigated the influence of physical activity on BMD in post and pre-menopausal women (n = 142, mean age of 50 years), participated in a clinical intervention trial for a period of 4 years. Exercise group (n = 80) participated in 45 minutes of physical activity per session (dancing, walking, and jogging), three days a week to reach the target heart rate (THR) for each subject. BMC and width of bilateral radius, ulna, and humerus were measured 11 times over the study period. Two groups were similar in terms of menopausal status and dietary intakes of 12 nutrients out of 15 measure nutrients, but exercise group had more intakes of calcium, magnesium than controls. During the study period, the exercise group lost significantly less BMD from the right and the left radius and ulna ($P<0.01$) than did controls. Rate of decrease in left humerus BMC and width were also significantly lower in the exercise group ($P<0.05$), with no significant differences from the controls for corresponding indicators of the other humerus. Similar results were obtained when pre and postmenopausal women were analyzed separately, suggesting that the effect of physical activity was not influenced by menopausal status. Briefly, this study showed that the effect of exercise intervention on bone loss for two hands was not similar and was not influenced by menopausal status. Clearly, such activities in which subjects are using less from their hands may be less effective on any change in bone density of forearm bones and differences between two hands may be related to the use of each hand.

Regular physical activity is also associated with bone health. Gregg et al (241) published a prospective study in 9704 women, aged 65 and older for a mean period of 7.6 years. Participation in 33 physical activities during the past year was assessed by a self-administered questionnaire, and then total physical activity index was scored and expressed in kilocalories per week. Women in lower quintiles of physical activity were older, were more likely to smoke and had lower self-rated health status and lower calcium intakes than women in higher quintiles ($P<0.05$). Activity was also positively related to the calcaneal and distal radial BMD ($P<0.001$). Likewise, the risk of hip fracture was lower in more active women than in less active ones. Multivariate adjusted RR for hip fracture among women with highest physical activity index (>2201 kcal/week) was 0.64

(95% CI 0.45-0.89 $P<0.003$), compared with those, who were less active. In contrast, sitting more than 8 hours per day was associated with an increased RR of hip fracture (age adjusted RR = 1.43, 95 % CI, 1.12-1.82) compared with who sat less than 6 hours per day. The relation between physical activity and hip fracture risk in this study was a dose-response relationship. Heavy household activity more than ten hours per week was also associated with a reduced RR of hip fracture (RR=78, 95% CI, 0.62-0.99 compared with women who reported less than 5 hours per week). Total physical activity, hours of household chores per day, and hours of sitting per day were not statistically significantly associated with wrist or vertebral fractures. Briefly, physical activity was found to be related to hip fracture risk with a dose-response relationship. This association was site-specific, with no relation to the risk of vertebral and wrist fracture, although it was positively associated with increasing the BMD at radial and calcaneal bones.

However, others reported different results. Greendale et al (243) published a cross-sectional study in 1703 men and women aged 50 years or more. Recall of usual exercise during teenage years, at the ages of 30 and 50, dietary intakes, BMI, alcohol use, medical history, and smoking were ascertained by standardized questionnaires. The usual pattern of physical activity in the last year considered as current physical activity. Based on the current and past physical activity, subjects were scored and categorized as strenuous, moderate, mild or less than mild exercise groups. There was no association between physical activity at any age with the risk of osteoporotic fracture in both men and women, although BMD at total hip was significantly increased by current exercise in a dose-response fashion ($P= 0.001$). The average bone mineral density at hip in strenuous exercisers was 6.5 % higher than that of mild or less than mild exercisers and 3% higher than moderate exercisers ($P= 0.09$). Current and past physical activity was not associated with bone density at the ultradistal radius, mid-radius and lumbar spine. Summing up, this study found no relation between exercise and hip fracture risk, in spite of a positive effect of physical activity on BMD at all hip sites. Clearly, recall bias may have affected the results of this study.

Physical activity was found to be protective for hip fracture in a case-control study from Hong Kong (155), in which 400 cases of hip fracture were compared with two sets of con-

trols: 400 community controls and 400 hospital in-patient controls matched with cases by 5-year age groups. The frequency of walking uphill and with a load at around the age of 35 were used as indices of past physical activity. Current and past physical activities were associated with reduced risk of hip fracture. Among current various activities, the highest OR was related to walking with load in women with less than once per day (adjusted OR= 2.3 95% CI, 1.2-4.7). Other activities were also significantly related to the risk of hip fracture in women except walking uphill. For men these associations were not statistically significant. Among past physical activities walking uphill and walking with load less than once a day were significantly associated with increasing hip fractures risk among men and women combined. When analyses performed separately, and after adjustment for smoking and alcohol consumption, walking with load (less than once per day) remained significant ($P<0.05$) in men and walking uphill (less than once per day) was significant ($P<0.05$) for women. In summary, current and past physical activity was protective for hip fracture risk but the effect varied with sex and the type of activity, with less effect in men.

Similar results were reported from Britain by Cooper et al (159). 300 hip fractured elderly men and women, aged 50 and over, were compared with 600 community controls, matched for age and sex. Five indices of current activities (during last six weeks) were derived: self reported walking speed, time spent standing indoors, time spent walking outdoors, frequency of loading activities (climbing stairs or carrying loads) and time spent in productive activities such as gardening and housework. Hip fracture risk among women was significantly associated with four indices of physical activity ($P<0.05$), namely, self reported walking speed, standing times, loading activities and productive activities. For men these associations were not significant. Adjustment for BMI, smoking, alcohol consumption, use of corticosteroids and history of stroke did not change the associations. This study again, found less pronounced effects in men compared with women and differences between different activities.

A population-based case-control study by Jaglal et al (244) examined the effects of past and recent physical activity on the risk of hip fracture in women. 381 hip fractured females aged 55-84 years were compared with 1138 randomised healthy controls, matched by 5-year age groups to the cases. Data collection from cases was performed 8-24 months

after hip fracture. Using a validated questionnaire, physical activities at ages 16, 30, and 50 years were ascertained, and then a physical activity score was calculated for each subject according to the reported frequency of participation in each activity. Based on theses scores, subjects were categorized as inactive, active and very active groups according to the values of lower and upper 20% of distribution in controls. Their recent physical activity was defined as activity in the past year for controls and activity in the year before fracture for the cases. Past high activity was associated with an adjusted OR of 0.54 (95% CI 0.33-0.88) compared with inactivity. Moderate activity was also associated with an OR of 0.66 (95% CI, 0.45-0.96). Adjusted ORs for recent activity was 1.15 (95% CI, 0.72-1.83) for very active subjects and 0.61 (95% CI, 0.41-0.90) for active subjects in comparison with inactive subjects. An interesting finding here is that reduced protection or none was afforded to women who were very active recently. Differences between cases and controls with respect to their health status and thus probability of recall bias, particularly for estimation of recent physical activity must be considered in interpreting the results. Recent physical activity may be over estimated in cases.

Keeping with the results of this study, O'Neill et al (245) reported possible hazards of vigorous activity in the elderly. 62 females with forearm fracture (aged 45-82 years) were compared with two groups of controls form the same catchment area as cases: 50 women who had fallen onto the hand, but without fracture and 116 women randomly selected from primary care age and sex registers. Walking at a brisk pace was associated with increasing the risk of distal forearm fracture. Compared with controls, cases who walked at a brisk pace were at higher risk of distal forearm fracture (OR = 3.5; 95% CI, 1.3- 9.6). Calcium intake, smoking, and alcohol consumption were taken into consideration.

Beside the hazards of vigorous activity, it may also pose a long-term threat upon elderly population. Silman et al (246) reported a significant association between heavy physical activity and vertebral deformity in older population. 14,261 men and women, aged 50-79 years, participated in this multi-central study across the Europe and their daily activity level during three times in their life span corresponding to the ages of 15-25 years, 25-50 years, and 50+ years was evaluated. After adjusting for age, centre, smoking, and body mass index, very heavy levels of activity in all three age groups were associated with an

increased risk of vertebral deformity in men (ORs ranged from 1.5-1.7, all were statistically significant). However, heavy physical activity posed no risk of vertebral deformity in women.

Physical activity maybe of benefit in young adults, during skeletal growth. Margulies et al (247) reported a prospective study in a cohort of 268 *young men* aged between 18-21 years.They measured BMC and bone width of tibia before and after 14 weeks of strenuous physical activity, including at least 8 hours strenuous training a day, six days a week. Physical activity was associated with 5.2% increase in BMC at right tibia (P <0.003) and 11.1% increase in BMC at the left tibia (P <0.0005). The width of tibia did not change greatly. These results show the beneficial effects of strenuous exercise activities on bone mass in young adults. This study however did not consider the effect of other confounders, particularly, nutritional variables. Additionally, no comparison was done to show the real effect of training on the changes of BMC. In the other words, it is not clear how much of increased values of BMC were due to natural growth or training program. Finally, period of 14 weeks is too short to show a real effect on bone density because median duration of remodelling period is 18 month.

The effect of teenage physical activity on hip fracture in the elderly was investigated by Nieves et al (202), who reported a case-control study in 229 women (161 hip fractured women and 168 matched controls) aged 45 and over. Heavy housework, fieldwork, heavy lifting or stair climbing, and participation in vigorous recreational activities during teenage years were ascertained and were used for creating an activity index for each person, according to weekly frequency of mentioned activities. Teenage physical activity was negatively associated with hip fracture risk. The adjusted OR for the highest quartile of *recreational* activity (4 activities per week or more) compared to the lowest quartile (<one activity per week) was 0.24 (95% CI 0.08-0.75). Total summary activity index was also associated with OR of hip fracture. The adjusted OR for the highest quartile of summary activity index (>5) compared to the lowest quartile (<1) was 0.63 (95% CI, 0.30-1.32). The ORs decreased significantly by increasing physical activity (P= 0.007).

2.3.3.1. Summary

In conclusion, studies of physical activity and bone health produced inconsistent results. In most studies, the associations between physical activity and bone health appeared to be site-specific and varied for different types of activities. This may be explained by differences in direction and the amount of forces posed to the skeleton in each specific activity. Also, it was found that men and women may benefit from activity, differently.

However, it is important to disentangle the effect of physical activity from that of other factors associated with activity. For example, only a few studies investigated the effect of physical activity on nutritional status and nutritional habits. This may partly explain some of the observed associations and discrepant results. Two studies in this regard showed positive association between physical activity and calcium intake (241;242), but for other nutrients, such evidence is missing. Furthermore, association between health status and physical activity is of particular importance and was ignored in most studies. Less active subjects are more likely to have poor general health and being older, with bad habits, such as smoking and high alcohol consumption (241). Finally, disentangle between physical activity and the effect of sun exposure and thus, vitamin D status is important, because most usual activities occur in out doors and may be associated with sunshine exposure and be confounded by seasonal variation in sunshine radiation and vitamin D status.

However, vigorous physical activity may be threatening. Some studies had pointed to the hazards of vigorous activities in the elderly (244-246), although others found inverse (241). It is important to note that the favourable effect of activity might be counterbalanced by the risk of falls, which may vary from one kind of activity to the other. Moreover, sudden strains on an already weakened bone my lead to fracture. Therefore, for elders it is important to be practically trained when are advised about the exercise.

Summing up, the effect of physical activity on bone health appeared to vary according to the skeletal site, sex, age and the type of activity, and it may be associated with nutritional intakes and particular lifestyle variables. Differentiate between the effects of other variables and that of activity is of particular importance.

2.4. Body size determinants and their importance for bone health

Relationships between body size and health outcome measures have long been inferred, mostly from epidemiological studies (156;230;248;249). In some part, the effects of body size might be related to its determinants during growth period in early life. Factors influencing body weight and height during growth may exert long-term or permanent effects on health.

2.4.1. Growth

Although the concept of growth is familiar to all of us, it is not easy to give a comprehensive definition. Growth is a term closely accompanies development, which means physiological improvement or specialization of an organ to perform its specific function. Although during the growth, an organism usually put on its size, growth is not necessarily increasing the size. In fact, the process of growth and development points to a series of events and changes to improve a living being from its earliest stages to the maturity (250). These events and changes consist of either increasing or decreasing in organ size (e.g. length increasing of long bones or degeneration the thymus gland after the age of six, respectively) or substitution of one tissue by another (as seen in substitution of cartilages by bone tissues during the growth). It may also include modification of some tissues for a better function (changing the glomerular cells from cubic to flattened form after birth for more effective filtration).

In human beings the major part and the maximum rate of cell replication occurs before birth (about 45 generations of cell divisions before birth in comparison with only five divisions after birth) (250;251). Most organs and systems are developed during this period of rapid growth, which is referred to as the "critical period". The timing of this "critical period" is different for different organs and systems, for example, the renal nephrons appear during the 5^{th} week (252) and the hepatic enzymes related to Glucose metabolism appear on weeks of 17 to 19 (253). In this stage, embryo is particularly sensitive to disruption by external factors. However, it does not mean that the programming of systems and organs accomplished only in prenatal period. Postnatal environmental factors are also of particular importance.

Major environmental determinants of growth in early embryonic life are nutrients, oxygen and hormones (254;255). The supply of nutrients and oxygen to the foetus is influenced by maternal nutritional state and her efficiency to supply her foetus, mother's body composition, and transport efficiency of utero-placental unit(251;253). Limitation on nutrient and oxygen supply may lead to decrease in cell proliferation during the critical period, in which organs are generating, or may scarify some organs to spare vital organs in order to save the foetal life, when organogenesis is established (254;255). Any change in substrate supply may also lead to alterations in endocrine status of the foetus, an effect that may last for a long time or the entire life (256;257). Besides the programming of several metabolic procedures, the trajectory of growth is also determined during this period, and thus body size may provide clues for the effects of early exposures on the growing foetus.

Evidence relating early life exposure and growth trajectory partly came from studies on babies who were small for gestational age. Studies on IUGR babies in Canada showed that these babies were more likely to be under-grown during postnatal growth (258;259). In a 4-year prospective study on 96 IUGR babies (258) it was found that at the age of six years, 35% of IUGR babies were still under-grown (defined by being at or below 3rd percentile of Stuart for height and weight), while only 8% of them reached above 50th percentile. Among normal babies matched to the study group, only 3% of babies were under-gown with 45% at or over 50th percentile. In another study (259), 29% of 158 small-for-date babies remained under-sized (below 5th percentile for weight and height) at the age of 2 years.

Walther and Ramaekers (260) have also reported that 52% of 25 disproportionately growth-retarded children permanently remained below 10th percentile of weight grow curve by the age of 3 years. 36% of these children remained below 10th percentile for height. In comparison with 25 controls, who were carefully matched for several variables, IUGR babies were more likely to remain under-grown, as 13 out of 25 IUGR and 2out of 25 controls were under the national 10th percentile at the age of 3 years.

Westwood et al (261), reported similar results. 33 full-term IUGR babies were compared with 33 matched controls and were followed-up until the age of 13-19. IUGR subjects were significantly smaller than controls at the ages of 13-19 years even after adjustment

for parental height and socio-economic status. These results have been supported by other studies (262).

These epidemiological studies indicate that the adverse intrauterine environment may affect weight and height in later life in spite of catch-up growth, seen in retarded children. Most children with intrauterine growth retardation tend to be small in comparison to the normal non-retarded children.

One may argue that the small body size seen in growth retarded children in later years, is due to adverse influences of infections and other diseases, which commonly seen in such children, or disadvantageous environmental factors during postnatal growth. Studies on IUGR children however, did not support this argument. In a study by Barros et al (262) while the risk of perinatal mortality in preterm babies (were appropriate for gestational age) was two times greater than small-for-date babies and their risk of being hospitalised for diseases such as diarrhoea and pneumonia during postnatal period was greater or equal to IUGR babies, their catch-up growth was more efficient in comparison with growth retarded children. Preterm babies are thought to be less or not affected by under nutrition in early pregnancy. In a case-control study (258) differences between AGA (appropriate for gestational age) and IUGR children in terms of height, weight and head circumference at ages 13 to 19 years were statistically significant even after controlling for socio-economic status. In the study of Walther et al (260), discussed above, cases and controls were matched for different environmental variables such as place of birth, duration of hospitalisation, feeding practice when leaving the ward, birth rank and social class. Differences in body size between AGA and IUGR in this study were independent of these variables. Likewise, it has been shown that for a majority of growth retarded children body size is significantly smaller than other children in their family who are assumed to be grown in the same environment (258). By 4 years of age 73% of IUGR children were reported to be shorter than their sisters and brothers with normal birth weight. These evidences are in accordance with the idea of programming of body size in early life.

Several physiologic bases have been explained for the associations seen between intrauterine exposures and body size in adulthood: permanent reduction in cell numbers during

the period of rapid cell proliferation may play a role as an adaptation to the limited supply (see above) and cell numbers limit ultimate body size (251).

Permanent alteration in endocrine profiles is thought to be another underlying mechanism. Lower levels of IGF-I and some other hormones such as insulin and TSH were shown in growth retarded babies as well as increasing levels of GH (growth hormone) and cortisol (263). All these hormones are known to affect the foetal growth. In this respect, cortisol and other corticosteroids may play a central role for the programming of growth (264). Besides of their effects on regulating the placental size and promotion of implantation, corticosteroids are important regulators of IGF-I and IGF-II and their receptors. IGFs are essential for foetal protein synthesis and regulation the distribution of substrates between maternal tissues, foetal tissues and the placenta, all are important for promotion of foetal growth(251;264;265).

Two other important roles of cortisol, which are essential for growth and many metabolic profiles in later life, are programming of several hepatic enzymes of fuel metabolism and programming of the hypothalamic-pituitary-adrenal axis (264;265).These regulatory effects of cortisol are lasting for the entire life. Although corticosteroids are essential for foetal development and tissue maturation in a normal range, the increasing amounts of cortisol, which may be produced as a result of maternal and/or foetal stress, due to foetal or maternal under nutrition, can permanently and adversely affect all these systems. High level of corticosteroids is also associated with growth retardation in a newborn.

Insulin is also associated with the growth via glucose utilization and protein synthesis (251). Colle, et al (266) in a study on IUGR children showed that the insulin response determined by raising of plasma insulin activity following glucose injection, was related to growth promotion in postnatal period. Growth retarded children at six month of age showed a lower insulin response in comparison to others who showed more efficient catch-up growth. They concluded that lower insulin production might be responsible for post-natal low growth rate seen in IUGR children.

However, postnatal and childhood exposures are also important and may affect the growth and thereby, adulthood body size. Childhood environmental factors including poverty,

overcrowding, nutrition and in general socio-economic class and childhood illness are among important determinants of adult height. It is consistently reported that adult height is determined by social class as the mean height of people from higher social classes were found to be significantly higher than that of people within lower social classes (500;501). Similar association between social class, poverty and parental nutritional state with body size at birth is now well documented and it is believed that the environmental exposures during early life and childhood development is translated into body size in later years and underlies the associations between body size and the risk of chronic diseases in the elderly (272;499;502). Adult stature, and to a lesser extent body weight, are strongly associated with their values in childhood (267;268), a phenomenon called tracking (41;251;253), which refers to the tendency for subjects who are small in relation to their peers of the same age to remain small, and vice versa. High correlation coefficients between body height and weight in childhood and adolescence have been documented in several studies. Power et al (269) in a study on a British birth cohort showed that the body height in adults is well predicted from childhood as the correlation coefficient between height at ages 7 and 33 y was found to be strong (r =70) for both sexes. Cooper et al (40) in their study on 413 men and women aged 63-73 years reported such tracking for body weight. In this study weight at 1 year was related to adult weight (r = 0.22 in women and r = 0.30 in men $P<0.001$). These results were replicated in another study on women in the UK (41), in which weight at age 1 and 5y were significantly related to the weight at age 21 (r = 0.35 and r = 0.67 respectively, $P<0.001$). Both studies also showed that the adult height is well predicted by weight at age 1y (with correlation coefficients of 0.41 among men in the former study and 0.39 for women in the latter). Similar results were reported by Gunnell et al (268). In this study height and weight in the age of over 65 were found to be well predicted by their relative values in childhood. Correlation coefficients between height in the elderly and height in the age of 5-7 were 0.84 and 0.77 in men and women, respectively.

Being tracked from childhood to the elderly, body size represents an important marker of the intrauterine and early postnatal exposures. Such exposures are important for programming of hormonal and metabolic systems for the entire life. Therefore, body weight

and in particular, height are indicators of the effects of early determinants of programming phenomenon for setting many hormonal and metabolic systems, affecting physiology and structure of human, thereafter.

However, beside early and late environmental influences on body size, adult body size has strong genetic components. A twin study of 586 monozygotic and 447 dizygotic twins (men and women), aged 18-81 years found the major part of variance in height, weight and BMI can be explained by genetic influences (498). Heritability of height ranged from 0.89 in the youngest group to 0.87 in the oldest and similarly, heritability of weight ranged between 0.86 in the youngest group to 0.70 in the oldest. Corresponding figures for BMI were 0.82-0.63. Rather similar results were found in a population-based study, in which mid parental height (the average of father's and mother's height) was used to predict offspring adult stature (455).

In conclusion, although body size is used as an indicator of childhood living condition due to its environmental component, it has a strong genetical component, which should be accounted for when associations between body size and chronic diseases are of interest.

2.4.2. Early nutrition and bone health

The effect of early nutrition on health in elderly has received much attention in last two decades since early evidence of association of foetal growth impairment with the risk of cardiovascular diseases in later years was developed by Barker and colleagues (277). Recent epidemiological studies suggested that early exposures are related to the risk of several chronic diseases in the elderly, such as coronary heart disease, cancer and diabetes (278;279) and accumulating evidence is still emerging in this respect. Such associations have been also suggested between early nutrition and bone health in the elderly (42).

Cooper et al (41) published a study, in which 153 females were traced for about 21 years from early infancy. Body weight at 1 year was significantly associated with BMC of spine and femoral neck (r = 0.32 and 0.26, respectively, $P<0.01$) at age of 21, independently of adult weight and BMI. Though associations between body weight measurements before age one and current BMC were not significant, after age one until age 21 became slightly stronger. BMD at both sites was not related to body weight at age one but became

stronger as age increased until age 21, which was apparently significant (r =0.29 and r =0.28 for spine and femur respectively, $P<0.001$). Weight at age one was a strong predictor of adult height (r = 0.39, $P<0.001$), suggesting that the trajectory of skeletal growth as a determinant of bone mass in adults is established early in life. However, it should be noted that BMC is a measure of bone mineral content that shows the amount of bone mineral in a skeletal unit (g) or a certain length (g/cm). The former is measured by dual energy absorptiometry and used for vertebra for example, or the whole body and the latter is measured by single energy absorptiometry and used for long bones (e.g. limbs). Bone mineral density (BMD) on the other hand, is a size adjusted index of bone mineral and is obtained by dividing BMC by the area of the scanned bone envelope (its unit is g/cm^2). For single energy techniques this area is equivalent to the distance between the opposing bone edges (bone width, cm); for dual energy measurements it is the designated bone area (bone area, cm^2). BMD is used in order to minimize measurement errors connected with positioning, movement and bone edge detection, and to make some adjustment for size differences between individuals (503). However, BMD is a mathematical construct and is not a true density measurement. It represents only a partial correction for bone size depending on the skeletal site. Both BMC and BMD are subject to confounding by skeletal size and failure to adjust for the skeletal size and bone area may lead to spurious or inflated relationships with other variables that are themselves related to size, such as dietary intake, obesity and energy expenditure (503).

Similar results were reported by another cohort study (40). Birth information and weight at 1 year were obtained from NHS documents for a cohort of 413 men and women aged 63-73 years from Hertfordshire. In this study, weight at one year was associated with adult BMC at spine and hip in women and with BMC at spine in men during the seventh decade of life (men: spine r = 0.16, P = 0.02; hip r = 0.06, P = 0.41, women: spine r = 0.15, P = 0.04; hip r = 0.15, P =0.03). These relations disappeared when were corrected for the bone area (BMD). Birth weight was not related to BMC and BMD in both sexes. This study showed that the bone mineral content (but not BMD) in adults is related to growth in infancy. Associations between body weight at age one with adult height and weight were similar to the results of the study described above. Adult height was strongly

related to weight at birth and one year in men and women (men: birth weight r = 0.28, one year r = 0.41, $P<0.001$ and in women: birth weight r = 0.15, $P<0.05$ one year r = 0.33, $P<0.001$). Corresponding associations for adult weight were less strong and for BMI no association was observed. Age, social class, smoking, alcohol consumption, calcium intake activity level and years since menopause for women were taken to account. Authors concluded that adult skeletal size as a determinant of BMC (bone mass depends on size and density of bone) is programmed during early years of life.

Given the relations between early life and bone mass later in the life, it is of importance whether there is any relation between early growth and fracture risk. Cooper at al (280) reported a study of a cohort of 3639 men and 3447 women, who were born and still lived in Finland, with their birth information and an average of 10 (SD = 4) body size measurements during childhood being available. This cohort was followed-up until maximal age of 71 years (165404 person-years) for the occurrence of hip fracture, which were obtained from the national hospital discharge register. While there was no association between any single measurement of body size and the risk of hip fracture, the rate of growth in childhood (ages between 7-15 years) was a strong predictor of hip fracture in both sexes. Subjects within the lowest quartile of the distribution of growth rate (of weight and height) were at a double risk of hip fracture in comparison to those in the highest quartile (hazard ratio = 1.9, 95%CI, 1.1- 3.2). Associations remained significant after adjustment for age and sex ($P<0.02$). The rate of change of BMI however, was not significantly related to hip fracture in later years. Possibility of selection bias (the sample was selected from those who born at hospital), neglecting possible effects of life style variables, nutritional variables and other confounders, which are likely to vary during this long period of time, particularly during world war II, were among the main limitations of this study.

The presented studies have documented that early nutrition and growth is determinant for bone mass and the risk of hip fracture in the elderly. Such relationships are thought to be related to a phenomenon called "programming", by which environmental stimuli during a critical period may cause persisting structural and functional changes in foetal systems and organs affecting through the life thereafter, suggested by professor Barker from university of Southampton (254). It is now evident that different foetal tissues and organs

have different growth and developmental time and, therefore, an environmental stimulus at a particular time, in which an organ or system is developing, may persistently affect the physiology, structure and the metabolism of that growing organ (251). Although our knowledge about the precise timing and mechanism of this process is still very meagre, there are many well-established examples of programming on animals and human. For example: in early pregnancy that may be the critical period for gonadal development, administration of high doses of stilboestrol and ethisterone causes uro-genital anomalies in exposed babies (281). Another example of programming phenomenon in human is amblyopia, which is a permanent subnormal visual acuity in one or both eyes because of sensory deprivation in early childhood during the critical period of eye development. In spite of correction of causal refractive errors, this subnormal visual acuity will remain forever (282). Relationships between foetal nutrition and many metabolic activities in adult life have been suggested to be related to the programming phenomenon, such as glucose intolerance and diabetes mellitus, hypercholesterolemia, and hyperfibrinogenemia (256).

Several physiologic mechanisms are thought to underlay the relations between early nutrition and bone mass in the elderly. Programming of hormonal profiles is of particular importance and has received attention in several studies. Fall et al (39) from a study on 37 healthy men aged 63-73 reported higher median GH secretion in subject with higher weight at one year.

Plasma cortisol in the elderly was also found to be related to birth weight. Phillips et al (283) measured fasting plasma cortisol in 370 men aged 59-70 years whose birth records were available. They reported that plasma cortisol level in subjects whose birth weight was lower than 2.5 Kg was significantly higher than those with birth weight of more than 4.3 Kg. These associations were independent of age and BMI.

Associations between birth weight and intestinal calcium absorption have also provided evidence of the effects of early nutrition on bone metabolism in the elderly. Arden et al (188) published a twin study, in which 322 postmenopausal women were participated. Calcium absorption (measured by the stable strontium technique) and plasma vitamin D were measured and birth weight was recalled. Birth weight was inversely associated with plasma vitamin D($1,25(OH)_2$VitD) and fractional intestinal calcium absorption, independ-

ently of age, season and customary calcium intake. Women with highest quintile of birth weight had 16% lower fractional calcium absorption than those within the lowest birth weight quintile. Adjustment for a number of variables including; age, serum concentration of calcium, 25(OH)VitD, creatinine, phosphate and PTH did not change these associations but when the plasma level of vitamin D ($1,25(OH)_2$VitD) was included in the model, the association became non-significant, indicting that the association between birth weight and calcium absorption is partly due to the effect of vitamin D.

Early nutrition has also been shown to modify the effect of VDR genotypes on bone density of the femoral and lumbar skeletal sites (73) (see section 2.2.3).

2.4.3. Summary

In conclusion, early environmental exposures are determinative for occurring osteoporosis and related fractures, through setting hormonal, metabolic and structural profiles. This phenomenon is known as "programming phenomenon".

Tracking of body size from early childhood made it possible to evaluate the effect of these early exposures on bone health and disentangle it from other effects related to the current diet. Skeletal proportions are also important clues for the effects of different factors during different stages of growth. However, it is important to clarify the association between bone health in the elderly with early nutrition, monitored by the skeletal size and its proportions, and with current diet. Understanding of these associations is important for making nutritional policy for various subgroups within the general population, with different nutritional backgrounds, aimed at preventing of the major health burden of osteoporosis among older persons.

2.5. Genetics of osteoporosis

It is now evident that genetic plays a central role in osteoporosis and its related fractures. For the purposes of this thesis genetical influences on bone health are not of interest but being aware of involving variables in this regard is necessary for the precise evaluation of the matter.

Theoretically, genetic factors may play several important roles in the pathogenesis of osteoporosis and propensity to osteoporotic fractures. Genetics may be determinative for:

1) Bone mass, especially peak bone mass in adolescents.
2) Muscle mass and thereby influence the risk of fall.
3) Lean and fat body mass, which are shown to be related to bone metabolism (47;284).
4) Growth rate and thereby influence the skeletal size, which is determinative for bone mineral content.
5) Geometry of the skeleton, particularly at the neck of the femur (femur axis length), which is determinative for fracture risk.
6) Mediation of hormonal effects on bone by regulating their cellular receptors such as oestrogen, vitamin D and growth hormone receptors.
7) Bone quality in terms of micro-architectural structure of the bone.

As has been previously discussed, osteoporosis as a multifactorial disorder is under influence of several variables, among which genetical factors play an important role. From a genetical perspective, osteoporosis is considered as a polygenic condition, in which several genes are involved and may be modulated by hormonal, environmental, and nutritional factors.

Most evidence of genetical susceptibility to osteoporosis came from family and twin studies. Family studies are looking for similarities within families or across successive generations for a trait (e.g. low bone density) and twin studies are looking for the resemblance of a trait between twin pairs. Twin studies are based on the fact that monozygotic (MZ) twins share 100% of their genes and thus will be the same for genetically determined traits. Their differences could be attributed to the environmental differences or measurement errors. Dizygotic twins (DZ) share half of their genes and, therefore, their difference in a trait could be attributed to both environmental and genetical differences (285). Heritability of a trait can be estimated from interclass correlations. If for example, correlation coefficient for a trait is significantly higher in MZ twins in comparison with DZ twins it can be concluded that the trait may be of genetical origin. When a trait is purely genetical, it will be expected that the interclass correlation for that trait among MZ twins be doubled as of that of DZ twins. For a polygenic trait the assumptions are that the effects of various genes are additive and shared environmental and total phenotypic variance is

similar for MZ and DZ twins. Violation of these assumptions may lead to over or under estimation of the heritability of a trait (77;285). However, twin studies may be subject to overestimation, because of sharing environment for the twins. It is very rare that two twins being separated after birth. Family studies may also be subject to similar violation (77). It is worth noting that in most genetical studies statistical association between a genotype and a trait is considered as causal relation whilst it may be only a coincident association. Full explanation of the advantages and limitations of these methods can be found in related textbooks and is out of scope of this thesis.

In spite of differences between studies of heritability of bone health, most studies demonstrated that bone density at a number of skeletal sites has a strong genetic component. Arden et al (286) published a twin study in 128 identical and 122 non-identical female twins (mean age of 60years) and reported a strong genetic component at a number of skeletal sites. Interclass correlation coefficients for BMD among MZ pairs ranged from 0.67 to 0.87 and among DZ twins ranged from 0.27 to 0.45. From these correlations the authors estimated a heritability of 0.46 to 0.84 for all measured skeletal sites including the lumbar spine, femoral neck, Ward's triangle, total hip, mid and distal forearm and the whole body. Body weight, HRT use, HRT duration, and years since menopause were taken to consideration. Hip axis length had also a strong genetic component (a height- adjusted heritability of 0.60, 95%CI, 0.18, 1.02).

Family studies have also demonstrated a strong genetic component for bone mineral density at several skeletal sites. Lutz et al (287) reported a family study on 37 healthy postmenopausal mothers (mean ages of 52 years, SD = 7), who were paired with their daughters (mean age 25 years, SD = 4). BMD was measured at lumbar spine and three femoral sites. Controlling for nutritional intakes, bone density at most maternal skeletal sites were highly correlated with their daughters. Separate analyses for post and premenopausal mothers showed stronger correlations among premenopausal compared with postmenopausal women, suggesting that genetic factors are important determinants of both bone gain and bone loss, which may act differently.

Racial differences are also of particular importance and may point to the genetical component of bone health. Black and white populations have been found to be at different risk

for osteoporosis. A study of black and white postmenopausal women showed a significant racial difference in BMD of most skeletal sites (288). 216 black and white women, aged 51 and over, were compared for BMD at midradius, lumbar spine, trochanter and femoral neck. After adjusting for age and weight by covariate analyses, black women had greater BMDs than white women at almost all measured sites ($P< 0.001$), except at trochanter ($P = 0.18$).

Similar differences were also reported among men (289;290). Barondess et al (289) published a study in 42 white and 37 black men aged 33-64 years, in which whole body BMC and BMD were compared between the two groups. Black men had significantly higher BMD and BMC than white men, with a 15% higher BMC ($P < 0.0001$) and 8% higher BMD ($P = 0.001$) than the white men.

Another study in this regard, produced similar results (290). Comparison between 59 white and 40 black men (aged 20-50 years) for BMD of the lumbar spine, trochanter, femoral neck and midradius showed a highly significant racial effect on BMD at all these sites, with lower values for whites.

Besides differences between black and white races, different non-black populations are also at different risks for low bone mass depending on their ethnicities, as for example, African-American women have higher bone density than Caucasian women (291).

In some part, racial and ethnic differences in bone mass may be explained by variations in body size or other confounders (292;293) but these could not be accounted for the totality of the ethnical differences (291). Variation in peak bone mass may provide another explanation for racial differences in bone density. Bachrach et al (294) from a 4-year prospective study in 423 healthy Asian, black, Hispanic, and white males and females (aged 9–25 yr) reported significant different levels of peak bone mass in blacks and non-blacks. BMD and volumetric bone mineral apparent density (BMAD) of spine, femoral neck, total hip, and whole body, measured annually, were consistently greater in blacks compared to the corresponding values in non-black subjects. Among non-blacks, differences in BMD were significant at some skeletal sites depending on the ethnic group and sex, for example, BMDs at femoral neck and the whole body were significantly different among Asians,

Hispanics, and white females, for which Asians had significantly lower values. A few differences were also observed among non-black male subjects. Similar to this study, Gilsanz et al (295) have reported about 11% higher vertebral bone density and 6% higher femoral cross-sectional area among 80 black children than their matched peers. Children aged 8–18 years and were matched for age, gender, height, weight, and stage of sexual development, using computed tomography.

This racial difference in bone mass seems to be tracked through adolescence to the elderly, as demonstrated by studies in childhood (295), in adulthood (296) and in advanced ages (288).

Similar to bone mass, osteoporotic fractures may also be affected by genetic factors. This was investigated by a prospective study (297), in which 2308 MZ and 5241 DZ twin pairs (a total of 15,098 subjects) at the baseline were followed-up for 25 years for the occurrence of osteoporotic fractures. The pairwise concordance for osteoporotic fracture was 9.6% (95%CI, 6.2%, 14.2%) in monozygotic pairs and 5.9% (95%CI, 4.0%, 8.4%) in dizygotic pairs. Men were more affected by genetic factors than women as the pairwise concordance rate for osteoporotic fractures in women was 9.5% (95%CI, 5.3%, 15.5%) in monozygotic pairs and 7.9% (95%CI, 5.2%, 11.4%) in dizygotic pairs and in men corresponding figures were 9.9% (95%CI, 4.4%, 18.5%) and 2.3% (95%CI, 0.6%, 5.7%), respectively in MZ and DZ pairs. Theses results are indicating that osteoporotic fractures may be modestly influenced by genetic factors, particularly in women.

A family study also investigated the effect of genetic on osteoporotic fractures in 1003 Caucasian women aged 45-64 (298). Among those with family history of osteoporotic fractures (hip and/or wrist fractures in their first-degree relatives) BMD was significantly lower than those without family history ($P<0.02$) and a higher risk of fracture (total risk for osteoporotic fractures was 2.02, 95%CI, 1.02, 3.78). Adjustment for age, BMD and BMI did not change the associations. Subjects with positive family history of osteoporotic fractures were also at higher risk of osteoporosis at spine and hip, although the trend for the latter was nonsignificant (OR for spinal osteoporosis = 1.82, 95%CI, 1.08, 3.05, P =0.02 and for hip = 1.72, 95%CI, 0.63, 4.71, P =0.29). The risk of fracture for family history group was site-specific, as subjects with family history of wrist fracture were at a 4-

fold risk of wrist fracture with no increased risk of spinal fracture. Furthermore, the risk was still significant after being adjusted for BMD, suggesting that the familial influence may act through other components such as muscle strength, the risk of fall, and/or bone structure.

Similar to this study, Cummings et al (226) in a prospective study of a cohort of 9516 women aged 65 and older reported a significant family association for hip fracture. Over a period of 4.1 years, using multivariable age-adjusted analyses, a maternal history of hip fracture doubled the risk of hip fracture (RR = 2.0, 95%CI,1.4, 2.9), and the increase in risk remained significant after adjustment for bone density. Dietary intakes, physical activity, smoking, alcohol use, menopausal status, medication and weight of the age of 25 were taken to consideration. Similar results have been reported by others (299;300).

Contrasting to the presented studies, Garnero et al (301) published a study of 120 twins, who aged 45-69 years and were on average 12 years after menopause. Using interclass correlations for bone formation and bone resorption markers, they concluded that a major part of bone resorption markers in postmenopausal women is not genetically determined. Bone formation markers (bone ALP, osteocalcin and C propeptide of type I collagen [PICP]), which exhibited only a slight increase after menopause, had a strong genetic component (heritability of bone ALP was 0.64 95%CI, 0.03, 1.25, for osteocalcin was 0.37, 95%CI, -0.11, 0.85, and that of PICP was 0.99,95%CI, 0.51, 1.47), whereas bone resorption markers (total D-pyridinoline, urinary type I collagen cross-linked peptide [NTX] and CrossLaps), which increased markedly after menopause, were less likely to be determined genetically (genetic associations were not significant for most of them except that of NTX, which was 0.55 95%CI, -0.02, 1.11). These data suggest that the contribution of genetic factors in bone turnover in postmenopausal women is likely to be small.

The results of epidemiologic studies spawned further investigations aimed at recognition of responsible genes for the occurrence of osteoporosis. As has been described, osteoporosis is generally considered as a polygenic disorder, which means there is no a single gene affecting the skeleton. In this regard, different studies have suggested different alleles as influential genetic factors. Transforming Growth Factor-β1 (TGFβ1) (302-304),

VDR, oestrogen receptor gene (77) and apolipoprotein E (305) are among such suspected alleles. Polymorphism of collagen type I (encoded by the COLIA1 and COLIA2 genes) has been also reported to be important for bone density (292), although others reported no relation (306). However, it is beyond the scope of this thesis to present these alleles in detail.

2.5.1. Summary

In conclusion, there is clear evidence of heritability of bone characteristics such as skeletal size, bone density, bone mineral content, bone turnover markers and fracture rates. Bone mineral status in all stages of the life is under major influence of genetic factors, from childhood to the elderly, and estimated heritability of bone mineral density is about 0.46 to 0.84, depending on the skeletal site measured (286). Genetic factors appear to be far more important than the combination of nutritional, hormonal, environmental and lifestyle factors in the pathogenesis of osteoporosis. Genetic factors are interacting with hormonal factors, diet and lifestyle variables and failing of most studies to consider these interactions may be a reason for discrepancies seen in the results. Moreover, modifying effect of other variables particularly those of early exposures during pre-and/or postnatal periods, have been neglected in almost all studies. Such effects have been discussed in previous sections. Current diet may also modify the phenotypic expression of some genetic propensities (148), suggesting the importance of considering environmental effects on the function of genes in the body and meanwhile it may introduce hope for improving health even in a specific genetical background.

Violations of the assumption of genetic studies especially gene-gene interactions might provide another explanation for discrepant results. Such interactions are difficult to be addressed, especially, when a condition is due to the effects of a number of genes. However, understanding the effects of genetics on bone health and the risk of fracture is likely to lead to effective preventive programs. Furthermore, understanding the genetical effects on variations in bone mass and osteoporosis may lead us to expect how other variables, including nutritional ones, may explain the variance of bone mass. Discrepancies of nutritional and other studies on osteoporosis may be explained by the effects of genetical factors.

The final important point is that the effect of genetics on bone, similar to those of other variables, is site-specific and may vary between men and women.

2.6. Markers of bone metabolism

2.6.1. Introduction

Bone metabolism is reflected in the plasma by a number of substances, including enzymes and newly synthesized or breakdown products of the bone matrix. These products, which are usually referred to as biochemical markers of bone turnover, are released into the plasma and can be measured in blood and/or in urine. In comparison with bone mass measurements, which are usually measures of a single skeletal site, and, therefore, may not represent other sites, biochemical markers can provide an index of the metabolic activity of bone tissue throughout the skeleton (307).

Classically, the biochemical markers are classified into two main groups, according to the metabolic process they are considered to reflect: bone formation or bone resorption. Table 2.6 summarises the key characteristics of the most important bone markers. Different clinical uses have been described for biochemical markers such as assessing bone tissue activity, screening of bone-involvement in diseases e.g. cancers, (308) monitoring treatments with bone-affecting drugs (309), prediction of fracture risk and bone loss in osteoporosis. Their advantages and limitations must be taken to consideration, when using these markers in practice. Using bone markers may have some advantages in practice including: being non-invasive, feasible, inexpensive, repeatable and representing the entire skeleton rather than a single skeletal site (310). Their limitations are: they may be affected by body clearance rate and some non-skeletal diseases, they are not disease-specific although reflect general metabolic disturbances of the skeleton (308;311;312). Presenting the details of practical usages and specifications of all markers is beyond the scope of this thesis.

In this section, published literature regarding to the implications and discriminative power of alkaline phosphatase (ALP), which has been traditionally served to mark bone formation, will be presented.

Table 2. 6- Markers of bone metabolism.

Marker	Source	Analytical Specimen	Specificity
Bone formation markers			
Total ALP	Bone, liver, intestine, placenta	Serum	Specific for bone formation only in the absence of hepatic diseases
Bone-specific ALP	Bone	Serum	Specific to bone tissues with about 20% cross-reactivity in some assays
Osteocalcin (OC)	Bone, platelets	Serum	Specific to osteoblasts, with some immunoreactive forms in the blood
Carboxy-terminal propeptide of procollagen-I (PICP)	Bone, soft tissue, skin	Serum	Specific to proliferating osteoblasts
Bone resorption markers			
Hydroxyproline	Bone, soft tissue, cartilage, skin	Urine	Not specific for bone
Deoxy-pyridinoline	Bone, dentine	Urine	Specific to collagen, with highest concentration in bone
Pyridinoline	Bone, cartilage, blood vessels, tendon	Urine	Specific to collagen, with highest concentration in bone
C-terminal telopeptides (CTX) of collagen I	Bone, skin	Serum	Derived from newly synthesised collagen type I, with highest contribution probably from bone
N-terminal-Cross-linked telopeptide of collagen I (NTX)	All tissues containing type I collagen	Urine, serum	Specific to collagen type I, with highest contribution probably from bone
Bone sialoprotein	Bone, dentine, hypertrophic cartilage	Serum	May be specific to osteoclasts

2.6.2. Alkaline phosphatase and bone health

Plasma alkaline phosphatase (ALP) is one of the bone formation markers, which is the most common marker of bone status in clinical use (307;313). It is an enzyme, seems to play an important role in the osteoid formation and calcification (see section 1.4.2), although its precise function is unknown (307;312;314). This enzyme is found in the

plasma membrane of active osteoblasts. However, ALP is not specific to bone tissue. In fact, total ALP (T ALP) consists of several isozymes derived from various tissues, such as liver, bone, intestine, spleen, kidney, and placenta. Bone and liver isoforms of ALP are the most abundant ones (307;312;314;315). Bone isoform of ALP (B ALP) is specific to bone and can be detected from liver isoform by different techniques, the most recent one is immunoassay technique. Electrophoresis, heat denaturation, precipitation and selective inhibition are other techniques (307;312). It is beyond the scope of this thesis to present these techniques in detail.

Close relationship between both T ALP and B ALP with bone health has been reported in several studies. For ease of interpretation and comparison, studies of B ALP and T ALP are summarised in Table 2.7.

2.6.3. Bone-specific ALP and bone health

In all studies of B ALP, presented in Table 2.7, increasing level of B ALP was associated with poorer bone health, defined as lower bone mass or increased the risk of osteoporotic fractures. In the study by Garnero et al (173) reported that the negative associations between bone mass measurements and B ALP strengthened by increasing the time after menopause. In all postmenopausal groups, defined as less than 20 years postmenopausal or between 20 and 30 years or over 30 years since menopause, this negative association was apparent, whereas in premenopausal women none of the associations was significant. Similarly, Khosla (319) found a negative association between B ALP and BMD at proximal femur in postmenopausal women and in men aged over 50 years ($r = 0.25$ [$P < 0.01$] and $r = 0.17$ [$P < 0.05$], respectively). This association was not significant in premenopausal women and in men under 50 years. In this study, B ALP was negatively associated with BMD at all measured skeletal sites (see Table 2.7) in both sexes (e.g. for total body $r = -0.21$ and $r = -0.42$ in men and women, respectively, $P < 0.001$). In a similar study (320), while B ALP was negatively associated with BMD at several hip and forearm sites in older people, there was no relationship between BMD and B ALP in a group of young men (n = 70, median age = 27 years), who were reserved as controls. In this study, after adjustment for a number of confounders such as age, BMI, serum albumin and creatinine, B ALP was negatively associated with BMD at several hip and forearm sites in older men.

These results indicated a possible modifying effect of menopausal status in women and age of around 50 for men.

Table 2. 7- Studies of plasma alkaline phosphatase and bone health.

Author	Subjects			Study design	ALP	Outcome measurement	Results
	n	Sex	Age(y)				
Garnero et al (173)	653	F	35-89	Cross-sectional	B ALP	BMD of hip, spine, radius, BMC of total body	Negative association in postmenopausal, no association in premenopausal women
Khosla (319)	650	M,F	21-94	Cross-sectional	B ALP	BMD of spine, radius, femur , total body	Negative association in postmenopausal women and men <50 y, no association in premenopausal women and men >50
Goemaere et al(320)	283	M	71-86	Cross-sectional	B ALP	BMD of hip, forearm	Negative association.
Dresner et al (321)	53	M,F	>65	Prospective (3-y)	B ALP	BMD changes at hip, spine	Negative association at hip, no relation at spine (refer to the text)
Ross et al (323)	512	F	69	Prospective (3-y)	B ALP	Osteoporotic fractures	Associated with fracture risk
Garnero et al (324)	435	F	50-89	Retrospective cohort (5-y)	B ALP	Osteoporotic fractures	Associated with fracture risk
Hulth (331)	100	F	63	Cross-sectional	T ALP	15 parameters of bone mass	Negative association with nine out of 15 parameters
Resch et al (333)	46	M	65	Case-control	T ALP	Spinal osteoporotic fractures	Higher T ALP in osteoporotic patients
Sudo, et al (334)	852	M,F	40	Cross-sectional	T ALP	Osteoporosis	Higher T ALP in osteoporotic patients
Glendenning et al (335)	32*	M,F	50	Cross-sectional	T ALP	Osteoporosis	Higher T ALP in osteoporotic patients
McKenna et al (138)	181	M,F	>60	Cross-sectional	T ALP	Plasma calcium and vitamin D	Negative association.

* Subjects were patients underwent cardiac transplantation

A further study by Dresner et al (321) reported that longitudinal bone loss at total hip (measured annually) was significantly associated with B ALP ($r = -0.38$, $P<0.05$), whereas, changes in BMD at lumbar spine were not related to B ALP. However, in this study, BMD in lumbar spine increased with age, indicating the possibility of sclerosis that may reflect a measurement error (322).

Ross et al (323) from the Hawaii Osteoporosis Study, found that B ALP can be used as a strong predictor of osteoporotic fracture risk. Age-adjusted OR for increasing one SD in B ALP was 1.54 (95%CI, 1.12, 2.12). Further adjustment for calcaneal BMD did not affect the OR, substantially, indicating that increased B ALP predicted the risk of osteoporotic fractures in postmenopausal women, independently of BMD. After adjustment for a number of bone-affecting variables by using multiple regression analysis, B ALP was still a strong predictor of osteoporotic fractures (P =0.017). Similarly, Garnero et al (324) reported a strong association between B ALP and fracture risk. In this study, RR of fracture for those with the highest quartile of B ALP in comparison with those within the lowest quartile of B ALP was 2.4 (95%CI, 1.3, 4.2). After adjustment for bone mineral density (BMD) of the hip, spine, radius, or total body, bone markers and hormones were still predictive of fracture risk with similar RRs. Predicting the risk of fracture independent of BMD, in these two presented studies may indicate that bone markers are partly representing bone mass. They may also represent the structure of bone tissue in terms of microarchitectural characteristics, which are important in bone strength and osteoporosis (325). Therefore, bone markers may be more comprehensive markers of bone strength, compared with bone mass measurements. However, this is remained to be fully investigated by prospective investigations. Current views is using of combinations of bone markers and bone mass measurements for more precise risk prediction (326-328) rather than single measurements (329;330).

2.6.4. Serum alkaline phosphatase (T ALP) and bone health

Table 2.7 presents the summary of studies of T ALP. Similar to B ALP, T ALP is found to be a strong predictor of bone mass and the risk of osteoporotic fractures. In this regard, Hulth et al (331) found that 9 out of 15 parameters of bone mass, including metacarpal cortical thickness, trabecular pattern of femur, gamma-ray absorptiometry of forearm, radiological density of spine and biopsy of iliac crest, were negatively and significantly related to T ALP (correlation coefficients,-0.17 to -0.33, P<0.01). However, associations between T ALP and three out of four parameters of spinal bone mass did not achieve to a statistically significant level. As stated before, for spinal bone measurements the possibility of measurement errors and their effects on the results should be borne in mind due to

bone sclerosis in vertebral bone margins of older persons or other possible artefacts (322;332).

Resch et al (333) reported that T ALP was significantly higher in spinal osteoporotic cases than controls (124.±7 vs.101.±6, $P<0.01$). Likewise, a study in Japan revealed that Plasma levels of T ALP were significantly higher in osteoporotic subjects than non-osteoporotic ones (334). They found a close correlation between T ALP and bone density in both sexes. Similar findings were reported in patients underwent cardiac transplantation (335) (106 ±15 vs.77 ±7 IU/L, $P<0.05$). In this study, however, the study sample was not similar to the general population, because of various clinical problems and using corticosteroids.

Furthermore, T ALP was shown to be negatively associated with plasma 25(OH)vit D and total calcium ($r = -0.22$, and $r = -17$, $P<0.01$, respectively) (138). T ALP was also significantly lower in vitamin D replete, defined as serum levels of 25(OH) VitD ≥25 nmol/L, in comparison with those who were vitamin D deplete (serum 25(OH) VitD <5nmol/L) or borderline vitamin D status ($t = 3.0$ and $t = 2.7$, $P<0.01$, respectively).

A further study in Japan noted lower plasma levels of T ALP in those who drunk a glass of milk or more, everyday (336). Age, BMI, drinking and smoking habits were taken to consideration. In this study, stratified analyses by five years age groups and being pre-or postmenopausal showed lower T ALP in milk consumers than non-consumers from both sexes. Similar results have been reported in children with low calcium intake (337). Calcium supplementation of 500mg/day in 30 children with low calcium intakes led to fall in mean T ALP within three months.

Clinical performance of T ALP has also been investigated in cancer patients with and without bone involvement (338). 153 cancer patients were matched with equal number of healthy subjects by age and sex. T ALP was significantly higher in cancer patients compared with healthy controls (median of T ALP in controls was 106 U/L vs. 145U/L in cancer patients, $P<0.0001$).Within the cancer patients subgroup, those with bone involvement, as diagnosed by radiographic and radioisotope bone imaging, had higher levels of T ALP, in comparison with those with advanced stages of disease but without bone

involvement (median of 168 vs. 145, $P<0.01$). These results indicate the valuable discriminative power of T ALP in bone involvement situations.

2.6.5. Comparison between T ALP and other bone markers

Comparison between T ALP and other well known bone-specific markers such as B ALP may be useful to verify the reliability of T ALP in the evaluation of bone metabolism in various situations. A few studies have addressed this issue by comparing bone biomarkers in various disease states, with or without direct measurements of bone status. Cosman et al (327) published a prospective study of 81 females (including pre and postmenopausal women) over a period of 3 years. BMD of lumbar spine and femoral neck measured every six months and bone makers including B ALP and T ALP were measured at the first BMD measurement. In this study, changes in spinal BMD was significantly related to both T ALP and B ALP, with rather equal correlation coefficients (r = -0.49 and -0.47, $P<0.001$, respectively). Corresponding correlations for changes in BMD of the hip were not significant for both markers.

T ALP has also been found to be strongly associated with B ALP in established osteoporosis. In a group of pre- and postmenopausal women (50 pre- and 93 postmenopausal healthy women and 111 osteoporotic patients with hip and vertebral fractures) (339), T ALP was measured by colorimetric technique and B ALP was measured by enzyme immunoassay (EIA). Both markers behaved similarly in relation to age and years since menopause: their changes due to age were very similar (r = 0.316 for B ALP and r = 0.319 for T ALP, P = 0.0001). Dividing subjects by the ten years since menopause, showed no significant difference in the concentrations of T ALP and B ALP among all menopausal groups. When the premenopausal women were age-matched with postmenopausal ones, both B ALP and T ALP were significantly higher in postmenopausal women than premenopausal ones (16.7 ± 4 vs. 26.3± 10.7 U/L for B ALP and 2.3 ±0.6 vs.3.1±1.2 for T ALP). Using Z-scores for osteoporotic fractured patients (calculated against postmenopausal women) showed no difference between T ALP and B ALP, indicating no difference in discriminative power between B ALP and T ALP. Correlations between T ALP and B ALP were calculated for the total of the subjects (n = 254), fractured women (n = 111) and healthy women (pre-and postmenopausal) (n = 143), separately. Corresponding

coefficients were 0.94, 0.94 and 0.93 (P = 0.0001), respectively. These results suggest that in relation to osteoporosis, there maybe no preference of B ALP over T ALP. In a similar study, Diego (340) reported a strong correlation between B ALP and T ALP in both osteoporotic patients and healthy controls. In this study, 42 osteoporotic postmenopausal women aged 62 years and 14 age-matched women were participated and T ALP was found to be strongly related to B ALP in both groups (r = 0.79, $P < 0.001$). Correlation coefficients in osteoporotic patients and healthy controls were r = 0.75 and r = 0.81, $P<0.001$, respectively.

Another study, conducted in a group of rather younger subjects, reported strong correlation between B ALP and T ALP (318). Among 80 men and women aged 21-51 years, correlation coefficient between B ALP and T ALP was r = 0.55 ($P<0.001$). In patients with Paget's disease this correlation coefficient increased to r = 0.94 ($P<0.001$).

Kushida (341) reported a study in a group of women and compared the clinical performance of T ALP with other bone markers in healthy pre-and postmenopausal women as well as vertebral osteoporotic patients. 95 premenopausal and 66 postmenopausal women were participated in this study. T ALP was significantly associated with all other bone markers, including resorption markers (Pyr, ICTP, and Dpyr) and formation markers (BGP and PICP), in the entire sample (correlation coefficients were from 0.2 to 0.5, P values from <0.05 to <0.001). Comparison between menopausal groups showed higher levels of T ALP in postmenopausal women compared with premenopausal ones (3.2 ± 0.1,vs 2.0± 0.1, $P<0.001$). However, in postmenopausal group differences between vertebral fractured subjects and non-fractured postmenopausal women were not significant. Correlations between T ALP and other markers, which are known to reflect bone metabolism, indicate that T ALP can be used as a reliable marker of bone metabolic activity in this group of women, although it may not be of use to diagnose those with osteoporotic spinal fracture.

In a study of patients with advanced cancers and with/without bone involvements, Piovesan et al (342), noted that T ALP and B ALP rose by parallel amounts and were highly correlated. In patients with lytic bone lesions and in those patients without bone involvement, T ALP and B ALP, measured by immunoradiometric assay, were highly correlated

(r = 0.67, P<0.001). Corresponding correlation in those patients with mixed bone lesions (lytic/and blastic) was r = 0.88, P<0.001.

Close relationship has also been found between T ALP and bone resorption markers. In a population-based study of men aged 71-86 (320), indices of bone formation including B ALP and OC were strongly associated with indices of bone resorption (r = 0.29 to 0.76, P<0.001) and all bone turnover makers were negatively associated with BMD (r = -0.17 to -0.34, P < 0.01). Similarly, Dresner et al (321) in a study of 53 healthy elderly men and women, aged over 65, found strong correlations between bone formation and resorption markers (r = 0.63 to 0.74, P < 0.01) during a follow-up period of 3 years. These results indicate that in postmenopausal women and elderly men, bone formation markers are coupled to bone resorption markers and, therefore, a marker that reflects bone formation is also predictive of bone resorption. Thus, bone formation markers give us an estimation of bone turnover, which is a major determinant of bone loss in the elderly (173;320;344).

Presented studies indicated the close relationship between T ALP and bone health. This, make it a reliable tool for monitoring bone loss and estimating fracture risk in populations (313), although it may not be of value for the diagnostic purposes in individuals with osteoporosis (316-318).

2.6.6. Factors affecting ALP levels

Different factors may affect the serum level of bone markers; most of them are affecting bone metabolism and have been discussed in previous sections. Some other factors may affect the serum level of alkaline phosphatase independent of bone metabolism. Age, sex, bone fracture, various diseases and different medicines are among such variables.

Age and menopause-Plasma level of ALP and other bone markers and their proportional concentrations are different at different stages of the lifespan, but the focus of this section is on the relations between bone markers and age in the elderly.

Several studies have investigated the relationships between age and bone markers. Takahashi et al (339) in a study of 143 pre-and postmenopausal women, stated above, found a positive association between B ALP and T ALP with age (r = 0.316, and r = 0.319, respectively, P<0.0001). Although menopause induced a significant increase in the plasma

level of both markers (an increase of 40% in T ALP and 55% in B ALP), there was no significant association between age and both markers when postmenopausal women were analysed separately. Furthermore, there was no difference in the plasma levels of both markers among postmenopausal women, who were divided by the time since menopause as 0-9 years, 10-19 years or ≥ 20 years. These results indicate that once markers have increased at menopause, they remain elevated and do not change with age.

Similarly, Garnero et al (318) in a study of 353 men and women aged 20-88 years, reported a linear correlation between B ALP and age in both sexes (r = 0.27, in men and r = 0.39, in women, $P<0.001$). These results were somewhat consistent with the study of Takahashi et al (339) in terms of correlation between age and B ALP in women, but separate analyses for pre-and postmenopausal women are not available from this study.

Khosla et al (319) in a study stated above, reported no association between age and B ALP in pre-and postmenopausal women and in men under or over 50 years, analysed separately. Although B ALP was not related to age, its relationship with BMD of hip influenced by menopause in women and the age of 50 in men. B ALP was related to BMD of the hip in men > 50 years and postmenopausal women (r = -0.17, $P<0.05$, and r = -0.25, $P<0.001$, respectively), while there was no significant association between B ALP and BMD at hip in men aged <50 years and premenopausal women.

Similarly, Garnero (173) in a study of 653 females aged 35-89, stated above, reported no significant correlation between age and B ALP in pre-and postmenopausal women. However, the relationship between BMD of the lumbar spine, the total hip and the distal radius, and BMC of the whole body influenced by age and years since menopause. Correlations between BMD and B ALP were not significant in premenopausal women, whereas, in postmenopausal women, with the time since menopause, associations between B ALP levels and BMD of different skeletal sites were increasingly negative.

In a further study, Romagnoli et al (345) reported no relationship between B ALP and age or years since menopause in a group of 277 pre- and postmenopausal women. In this study, menopause induced an increase of 39% in B ALP and 46% in T ALP.

In summary, all presented studies consistently reported increased levels of B ALP and T ALP in postmenopausal women in comparison with premenopausal women. In studies, in which pooled data for pre-and postmenopausal women were used, linear association between age and ALP was reported (318;339), while separate analysis for pre-and postmenopausal women found no significant association between age and ALP in both pre-and postmenopausal women (173;319;339). These results indicate that the relationship between age and ALP found in pooled data is related to the incremental effects of menopause on bone markers. This increase will remain consistent in years after menopause. More importantly, menopause was reported to modify the relationship between bone mass and bone markers as the association between bone mass measurements and bone markers were not significant in premenopausal women, while were increasingly significant by years after menopause in postmenopausal women (173). Similar relationship was reported in men as in pool data for a wide age-range, bone formation markers were related to age, while in separate analyses for elderly and younger adults revealed no association between age and bone markers in either younger or elderly men (319). In addition, association between bone markers and bone measurements were not significant in younger men whereas was significant in older men (319). However, strong relationship between bone measurements (BMD and BMC) in most measured skeletal sites and ALP was documented in most studies with some differences among various skeletal sites.

Gender-Increase of bone markers due to menopause is now well documented, as discussed in the above section. Parallel increase in concentrations of bone formation and bone resorption markers was shown in a number of studies, suggesting that increasing bone turnover in older people is a major determinant of bone loss the elderly. In this regard gender difference in the magnitude of the associations between bone markers and bone measurements is of importance. Generally, association between bone markers and bone measurements was reported to be much stronger in women than in men, especially in studies, in which men and women were compared (319).

Bone fracture- Prior bone fractures are important when serum concentrations of bone markers are measured. Bone fracture may lead to an increase of 11-78% in bone markers in the first 2-6 weeks after fracture (343;347;348). Ingle et al (347;348) investigated the

changes of bone markers in subjects with ankle and forearm fractures. Among 20 women with forearm fracture and age of 63 years, bone formation markers increased between week 1-4 by 13-52% ($P<0.001$) and remained elevated for 52 weeks. Bone resorption markers increased by 18-35% in weeks 2-6 and returned to normal by the week of 52. In a further study, among seven postmenopausal women and seven elderly men (age 63years), ankle fracture led to a significant increase of bone formation markers between week1-4 by 11-78% ($P<0.01$). B ALP returned to normal up to 52 weeks, while OC remained elevated at this week. It is noteworthy that many fractures such as vertebral fractures may remain asymptomatic, while affecting the serum concentration of bone markers. Therefore, it is important to determine whether the subject has had a fracture of any kind in the year proceeding to the measurement of biomarkers (347). Inactivity due to a fracture is also important, as inactivity may affect both bone health and bone markers (349).

Diseases- Most chronic diseases and subjects general health may affect bone health. But some diseases may cause an increase in bone markers, beyond the amounts reflecting bone involvement. Liver and renal diseases are such examples. Increasing the bone markers is due to contributions from non-bone sources or impairment of clearance mechanism. For ALP, it is preferred generally to measure bone-specific isoforms to estimate more specifically the skeletal-produced ALP, especially when liver disease is suspected. In such cases, false positive results are still possible, due to cross-reactivity, which may be 16-20%, even with the novel methods of immunoassay (307;312;318). Gradual impairment of the kidney in the elderly may cause increase in markers excreted by the kidney such as OC (307).

Physical activity- Both activity and inactivity may affect plasma levels f bone markers beyond their effects on bone. The acute effect of activity may be an increase of bone markers, which may persist up to 72 hours after 30-90 minutes of a usual activity (such as brisk walking) (350;351). However, association between exercise and bone markers is a matter of controversy. Some studies reported no effect (352;353), whereas others reported some changes in the plasma concentrations of bone markers due to activity (354). Ryan et al (352) reported that 16 weeks of resistive training caused no increase in B ALP and OC in postmenopausal women. In contrast, Swezey et al (354) reported an increase in bone

formation markers due to 8 weeks resistive isometric exercises, with no change in bone resorption markers (354). Similarly, 10 weeks military training in young men caused an increase of 13% in B ALP with no change in bone resorption markers (355). Likewise, 5 weeks summer school exercise program in boys of age 16 years reported to increase the serum level of B ALP by 21% and to decrease bone resorption markers, significantly (356). In a similar study in elderly men, 16 weeks of training exercise increased B ALP by 26% (357). Discrepant results may be due to differences in methodology in terms of the type of exercise, sample selection methods and the ability of subjects to perform exercises. However, alterations in bone markers due to long periods of activity is because of the effect of physical activity on bone, for which bone markers are representative, but it is important to minimize the acute effects of activity on bone markers. Such effects may lead to false positive or false negative interpretations of bone marker measurements. Therefore, it is important to be aware of any kind of activity in at least 24 h before to the measurement of biomarkers. It has been advised that subjects should be asked to refrain from exercise at least 24 hours before collection of samples for biomarker measurements (349;358).

Drugs- Consumption of some medications may cause rapid changes in biomarkers. Antiresorptive drugs, HRT, anticonvulsants, thiazide antidiuretics may increase or decrease the plasma level of bone markers up to 70% (309;349;358). It is therefore important to enquire about medication history of the subject before any bone marker measurement. It has been recommended that medications used for the treatment of osteoporosis or known to affect calcium metabolism be discontinued at least one month before bone marker measurement (314).

Seasonal variation- Seasonal variation in bone markers may account for more of the variability of most bone markers up to 20% (359). Similar variability in vitamin D status has also been noted in a number of studies, discussed previously (see section 2.1.3.2). There maybe a causality association. However, in clinical or population-based studies, it is important to consider the month of the measurements and the place, in which the investigation has been conducted, particularly in countries of the northern latitudes, such as the United Kingdom.

Other considerations- For most bone markers a diurnal variation in their activity has been reported (343;349), with an increase at night and decrease during the day. For B ALP a peak between 1100 and 1400 hours and another possible peak at 2330 hours has been mentioned (343). However, for T ALP it has been estimated that daily variation be less than 4%, which is not a major determinant in its biological variation (312). This variability is thought to be due to the circadian rhythm in the bone isoform. To minimise the effect of circadian rhythm on clinical interpretation of bone markers it is important to control the timing of sample collection. Furthermore, the level of ALP may increase after a fatty meal in persons of blood groups O and B (360;361), due to increase in the intestinal isoform of ALP, thus, it may be preferable to collect the samples in early morning after an overnight fasting, although it may not be essential in the variability of T ALP (314).

2.6.7. Bone biomarkers and osteoporotic syndromes

As stated earlier, there are two types of osteoporosis; type-I and type-II. The former is the dominant form in early postmenopausal women (20 years from menopause or less) and can be characterised by high bone turnover and rapid phase of bone loss (2-3%/year) and the latter is seen in late postmenopausal women (20 years after menopause) and aged men and in comparison with the type-I is characterised by lower bone turnover and slower bone loss (<0.5%/year). Since bone turnover leads to an increase in bone markers, the rapid-bone loss syndrome is expected to increase bone markers more than slow-bone loss syndrome. Comparison between these two syndromes showed that both bone formation markers (e.g. osteocalcin and bone ALP) and bone resorption markers were higher in rapid bone losers than slow bone losers (508). In osteoporotic subjects at a plasma level of 2SD of bone ALP above the mean, the probability of rapid bone loss was 80% whereas, at 2SD below the mean the probability of rapid bone loss was only 20% (508). In both syndromes bone resorption increases more than bone formation and this is reflected by bone markers and therefore, bone resorption markers will increase more than formation markers. The rapid phase of bone loss (type I osteoporosis) is associated with a normal or slightly decreased PTH secretion but the slow phase of bone loss (type II osteoporosis) is associated with a progressive increase in serum PTH level (506). The patterns of changes in PTH levels has been suggested to be used to differentiate the two types of osteoporosis

(506), although there is no enough evidence for a cut-off point of PTH in this regard. Summing up, proportional to bone turnover rate, bone biomarkers are expected to increase in osteoporosis type I more than osteoporosis type II. However, there are no accepted cut-off points for predicting bone fractures in these two types of osteoporosis.

2.6.8. Summary

Bone markers are direct or indirect markers of bone remodelling activity. They reflect different aspects of bone metabolic activity such as bone formation and bone resorption. They have been used to estimate bone mass, bone loss, fracture risk and bone response to treatments. Alkaline phosphatase is a well-established marker of bone formation, which is commonly used in clinical and epidemiological studies of bone metabolism, and plays important roles in bone metabolism. Presented studies in this review have documented the clinical relevance and the discriminative power of T ALP and B ALP and a few studies have compared these two indices with each other and with other markers of bone turnover to verify their reliability in different physiologic and pathologic situations.

Several studies have noted that both B ALP and T ALP are well correlated with bone mass measurements in older individuals (173;319;320;331;333;335). These associations were found to be modified by menopausal status in women and age in men, as were not significant in younger men and premenopausal women, while were significant in older men and postmenopausal women with increasing in magnitude by the years since menopause. These associations were also found to vary by sex and differ by the skeletal site, as they were much stronger in women than in men and were markedly weaker for spinal sites than for other sites. Also, B ALP was shown to be predictive for bone loss (321) and both markers were related to the risk of osteoporotic fractures in older population (323;324;333). Furthermore, these markers have been found to predict fracture risk independently of bone density (323;324). This may indicate that beside representing bone mass, bone markers may also represent the structure of bone tissue in terms of micro-architectural characteristics, which are important in bone strength and osteoporosis (325).

Most studies, which compared the two markers in different situations, have shown good agreement between B ALP and T ALP in either healthy or osteoporotic subjects. Strong

correlations between these markers and bone resorption markers suggest that these markers could be confidently used as markers of bone turnover.

However, these indexes of bone activity are influenced by a number of factors, independent of overall rates of remodelling. These are mostly practical points in measuring biomarkers and need to be considered to avoid misinterpretation of the results.

In conclusion, studies presented in this review illustrated the potential usefulness of the bone markers, and, in particular, ALP, in clinical and epidemiological studies. They can be of appreciable value to assess bone activity in different physiologic and pathologic situations. Likewise, in the absence of liver disease, T ALP may also serve as a valuable tool for monitoring bone formation and osteoblast activity, and, therefore, may be useful for bone research in the elderly (307;362). However, none of bone markers is disease-specific, and, therefore, may not be of benefit for diagnostic purposes.

2.7. Muscle weakness, Risk of fall and related variables

The risk of osteoporotic fracture is the function of bone strength and propensity to fall. The former is related to bone mineral status and its structure, discussed in the previous sections and the latter will be discussed here.

2.7.1. Fall risk and osteoporotic fractures

Falling is one of the most common risk factors for hip fracture. It contributes to more than 90% of the hip fractures that occur each year (363;364). It is also a common accident among the elderly population as 30% of the elderly persons fall each year (364). Therefore, fall prevention could be an important part of the fracture prevention programs.

Similar to osteoporosis, falls in the elders are multifactorial in nature. Generally, falls have two types of risk factors: intrinsic factors (patient-related factors such as muscle weakness, age and diseases) and extrinsic factors (e.g. environmental hazards, time of the day and hazardous activities) (365). These two types of factors contribute to the risk of fall, almost equally, as reported by Hale et al (365). This prospective study of 120 ambulatory geriatric outpatients (mean age of 75 years) found that 48% of falls caused by intrinsic factors and 50% were due to extrinsic factors. Similarly, Nyberg et al (363) reported a 47% contribution of extrinsic factors versus 24% of intrinsic factors to the occurrence of falling in 123 hip fractured patients aged 65 years or more (363). In this study, however, due to poor patients' descriptions, 22% of falls remained unclassified.

Several intrinsic factors may lead to an accidental fall. Balance impairment, cognitive impairment and muscle weakness were the major intrinsic factors in the study of Hale et al (365). Muscle weakness found to be resulted in fall in 11% of fallers (365). Several other intrinsic factors have also been reported by other studies, including medications, undernutrition and inactivity (99;364;366-369). These may lead to poor balance or muscle weakness. For the purposes of this thesis, literature regarding muscle weakness and its relation to the risk of fall and relevant effective factors will be reviewed.

2.7.2. Muscular function and risk of fall

One of the most important intrinsic determinants of falling risk is muscle weakness (370), which is particularly common in the elderly and may progress with increasing the age

(371). Muscle weakness is determinative for falls through interfering with balance and causing poor motor performance. Decline in muscle mass may also affect the protective mechanisms that reduce the impact of falling through the reduction of the protective layer of soft tissue-padding covered the skeleton (48;91).

The effect of muscle function on the risk of falling has been investigated by a number of studies. A study of 217 institutionalised and non-institutionalised elderly subjects (368) found that fallers had significantly more hip weakness than non-fallers in both groups of the study population. In addition, subjects, who suffer from hip weakness were at 8-10 fold increased risk of falling (e.g. OR for institutionalised subjects was 8.36, 95%CI, 2.71, 25.79, $P<0.001$). Considering a number of confounders by regression analyses, hip weakness was still the strongest predictor of falling during one-year study period.

A national survey in Britain (370) reported reduced grip strength as a strong predictor of fall among 983 elderly people. After adjustment for age, sex and area of residence, RR for fall among subjects within the lowest third of handgrip strength was 2.3 (95%CI, 1.5, 3.8) in comparison with those in the upper third. Weight loss, sedative medicines and low activity were other determinants of fall risk in this study.

In a further study (372), handgrip strength was the most influential variable affecting the risk of fall. In this study 1042 individuals aged 65 years and over were participated and a number of risk factors and anthropometric variables were taken to consideration by using discriminant analysis. Others have reported similar results (373;374).

Muscular strength is also a determinant for the risk of fracture during a fall. Luukinen et al (375) published a case-control study, in which 82 hip fracture cases (aged 70 years or older) were compared with the same number of controls, who had soft tissue injuries due to a fall, with no fracture, and were matched by age, sex and location of the first injury. Reduced muscle strength (measured by knee extension and handgrip strength) was associated with increasing the risk of fracture during falls, after adjustment for age, sex and a number of influential factors (OR for knee extension weakness = 3.38, 95%CI, 1.00, 11.4). Handgrip strength was significantly lower in fractured fallers than in non-fractured ones ($P<0.05$).

However, another study in this regard reported somewhat different results. Nevitt et al (374) reported a one-year prospective study of 325 elderly persons (aged 60 years and over), who had fallen at least once during previous 12 month. 10 Kg decrease of handgrip strength was associated with increasing the risk of minor injuries during a fall (RR = 1.5, 95%CI, 1.0, 2.3). The relative risk was adjusted for a number of racial, clinical, functional and anthropometric variables. In this study, however, handgrip strength was not a statistically significant predictor of major injuries including fractures when other variables were taken to consideration.

2.7.3. Factors influencing muscle function

2.7.3.1. Age

Sarcopaenia, the loss of muscle mass and strength, is considered as a function of age (376), although various factors such as low physical activity, genetical, hormonal and nutritional factors may play roles. Ageing is also accompanied by changing the fibre composition of muscles and the tissue composition. Muscle loss and composition changes can be resulted in decrease of muscle strength and predisposing to falls.

Age-related reduction of muscle mass and muscle strength has been well documented by several studies (377-380). It has been estimated that skeletal muscle mass (SM) declines between 0.5% to 1% annually, in men and women over 60 years (379;380) and muscle strength declines about 12%-15% per decade from the third to the eight decades (377). The result of such decrement is remarkable in the elderly population, as more than 30% of people of age over 80 suffer from sarcopaenia (381). In a study of 337 men and women aged 64 to 93 years, the prevalence of sarcopaenia, defined as skeletal muscle mass/height^2 (square meters) less than 2 standard deviations below the mean for young, healthy reference population, was found to be 23% in women and 27% in men (381). Among persons with age of 80 years and over, 31% of women and 53% of men were sarcopaenic. Arden et al also reported negative association between age and muscle strength (47) among 706 postmenopausal women aged 45-70 years. In this study, handgrip strength and leg extensor power were found to be negatively correlated with age ($r = -0.18$ and $r = -0.26$, respectively, $P<0.01$)

Using body potassium, others have reported rather similar reduction, particularly in ages of over 65 years (378-380;382). Aloia et al (382) reported a study in 233 black and white women. From the age of 20 to 70 years, SM decreased by 8% in black and by 22% in white women. Age was negatively correlated with SM in both races, although for blacks it did not reach to a statistically significant level (for black women, r = -0.14, $P = 0.18$ and for white women, r = -0.42, $P < 0.0001$).

There is evidence indicating that the relations between age and muscle loss has a curvilinear component (378;379;383;384). Janssen et al (384) reported a study, in which the distribution of SM was measured in 468 men and women aged 18-88 years. Total, upper and lower body SM in both sexes were significantly negatively related to the age (e.g. for total body SM men r = -0.24, women r = -0.29, $P < 0.05$). Multiple regression analysis showed a negative non-linear association between age and SM, with a markedly decrease in SM in ages around 45 years in both sexes. Decline of SM was started in the third decades. Another study of 51 healthy women aged 54-76 years (378) found also a curvilinear association between age and SM (defined as K/FFM), with accelerated muscle loss after the age of 65 years. In addition, it was found that muscle strength is also associated non-linearly and negatively to the age (383). Peak of handgrip strength was reached in ages of 25-34 in men and 35-44 in women.

In addition to changes of muscle mass, the composition of muscles undergoes substantial changes due to aging. Infiltration of fat and connective tissues results in less contractile tissue in muscles of older people compared with that of younger ones (385). The composition of contractile tissue is also subject to substantial changes by aging. The number and the size of type 2 fibres (fast-twitch) decrease markedly with increasing age, whilst the number and the size of type1 fibres (slow-twitch) are less likely to be affected by age, and therefore, the proportion of the fibres type alters with age in the favour of slow-twitch fibres (385). The overall result is less contraction capability of muscles among the elderly subjects, leading to poorer motor performance and propensity to fall.

2.7.3.2. Nutrition

The association between diet and muscle performance has not been studied extensively, although muscle mass measures (MAMA [mid-arm muscle area], and mid-arm muscle

circumference) have been widely used as nutritional indices for assessing the general nutritional status. Mostly, in muscle performance studies, anthropometric markers of general nutrition, such as BMI, weight and height have been used rather than nutrients or dietary intakes with the exception of few studies that focused on vitamin D.

A study of 94 men and 190 women aged 55 years and over from Malawi (392) reported positive correlations between handgrip strength and several anthropometric nutrition indices, including; weight, height, mid-upper arm circumference, triceps skin fold and arm span in both sexes ($P<0.001$). After controlling for potential confounders (sex, age and height), the association between handgrip strength and nutrition indices remained significant and positive in both men and women. Each nutrition indicator explained more than 10% of the variation in handgrip strength. Similar results for the association between muscle strength and nutrition indices have been reported by other studies (393;394). Most studies reported handgrip strength as a reliable index of nutritional status. Although in all studies handgrip strength was weaker in people with lower values of other anthropometric nutrition indices, none of them specifically addressed the relation between handgrip strength and dietary intakes. There are limited numbers of such studies, almost all of them focused on vitamin D nutritional status. In this respect, Mowe et al (399) in a study of 349 men and women (246 hospitalised and 103 home-living subjects aged 70-91 years), found positive correlation between handgrip strength and serum 25(OH) VitD (r =0.22, in hospitalized and r = 0.37, in home-dwellings, $P<0.001$). Similar correlations were found for other measures of muscle function including; proximal muscle function, general muscle fitness and accident falls. Multiple regression analysis revealed handgrip strength as the strongest predictor of serum 25(OH) VitD ($P<0.002$). BMI, arm muscle circumference, serum albumin and heart disease were entered as independent variables.

These results are consistent with another study of 319 elderly subjects (103 women, 216 men, aged 65-95 years) (400). In this study, both 25(OH) VitD and 1, $25(OH)_2$Vit D were significantly correlated with LEP (Leg extension power) in men (r = 0.24; $P = 0.0004$ and r = 0.14; $P = 0.045$, respectively). In women, however, only $1,25(OH)_2$Vit D was significantly correlated with LEP (r = 0.22; $P = 0.034$). Likewise, a study of 63 community-dwelling elderly women, mean age of 82.5 years (±5.4-y) with low serum 25(OH) VitD

concentration (<40 nmol/L) showed a significant association between vitamin D status with handgrip strength and walking distance (401).

Studies on vitamin D deficit patients showed that muscle weakness, as the main clinical feature of vitamin D deficiency, can be reversed by a proper supplementation (403-405). Verhaar et al (404) reported a study of 10 vitamin D–deficient (serum 25(OH) VitD<20 nmol/L) elderly women (mean age: 76 y), who were matched with 13 controls by age and sex, with normal vitamin D levels (serum 25(OH) VitD>30 nmol/L). In this study, muscle strength and mobility, measured by knee extension strength and walking distance, improved in cases by 6 months treatment with 0.5 μg α-25(OH) VitD/d, whereas no improvement was observed in control group who received no therapy. Similarly, a randomised, controlled intervention study of 37 homebound older persons with low vitamin D status, as indicated by serum 25(OH) VitD concentrations of less than 15 ng/mL, showed a significant beneficial effects for supplementation with vitamin D (405). In this study, supplementation with vitamin D and calcium significantly improved the "time taken to dress" and functional ability as measured with the Frail Elderly Functional Assessment Questionnaire. However, the results of studies of patients with gross deficiency may not be applicable to the general healthy population, with no manifestation of severe vitamin D deficiency.

Relationship between vitamin D and muscle strength seems to follow a threshold behaviour as in studies of subjects, who were vitamin D-replete, no association between plasma vitamin D or supplementation and muscle strength was reported (406;407). Such association is similar to those of other nutrients and health outcomes. However, in well-nourished and healthy elderly people decline of muscle strength is still inevitable, indicating that many other variables are likely to be in operation (371).

Some cautions, however, needed in interpretation of the presented results of vitamin D and muscle strength, because of neglecting the possible confounding by physical activity. Both vitamin D and muscle strength are strongly related to physical activity, higher activity can improve vitamin D status and at the same time improves the muscle strength, and, therefore, it is important to disentangle the effect of training or physical activity from that of vitamin D within normal distributions. In addition, even though the muscle weakness is

associated with poor vitamin D status, it may not indicate causality. Hyperparathyroidism may also contribute to the condition, which may be a feature of hypovitaminosis D and may be dealt by various interventions (402).

2.7.3.3. Physical activity

Association of physical activity and muscle mass and muscle strength was reported by a number of investigations (159;364;366;371;376;383).

Hansen et al (378) published a study of 51 postmenopausal women aged 54–76 y, and investigated the effect of physical activity on muscle mass, estimated by total body potassium (TBK). Based on the estimate of metabolic equivalent (MET) of habitual physical activity (household, occupational, and recreational), subjects were categorized as high (n = 25) and low (n = 26) activity groups. Women of high activity group had on average 6.5% more potassium per FFM (fat free mass) (K/FFM) than did their less-active counterparts ($P < 0.01$). They also had higher values of TBK ($P = 0.04$) and K/ht ($P = 0.02$). The two groups were not significantly different in terms of age, height, weight, or hormonal status. Using multiple regression analysis, physical activity was the major determinant of the potassium content of FFM ($P = 0.02$), considering activity group, age, estradiol category, and IGF-I bioactivity, simultaneously in the model. Together with age, physical activity was accounted for 21% of the variance in K/FFM. These results indicate that physical activity is a major determinant of muscle mass in the elderly, independent of other variables, known to influence muscles.

A case-control study of 900 persons (300 hip fractured men and women matched for age and sex with community 600 controls aged 55-84 years) in the UK reported strong association between physical activity and handgrip strength (159) (for details of the study see section 2.2.4). In this study, after adjustment for age and sex, all five indices of current activities (self reported walking speed, time spent standing indoors, time spent walking outdoors, frequency of loading activities and time spent in productive activities) were associated significantly with handgrip strength. The results of other studies are rather consistent with the presented studies, suggesting a preventive role of exercise for sarcopaenia in the elderly (364;366;371;376).

2.7.3.4. Body size and muscle strength

Body build as measured by body weight and height has been also found to be related to muscle function. In the study by Janssen et al (384) (see the above section) 50% of variability of SM in men and women was explained by weight and height. Height was linearly (in men r = 0.48 and in women r = 0.53, $P < 0.001$) and weight was curvilinearly related to the SM.

Gallagher et al (408) have also reported a positive association between body size and skeletal muscle mass from a cross-sectional analysis of 284 non-obese (BMI<36) men and women, mean age of 47 years. In this study, total, leg, and arm muscle mass, measured by dual-energy X-ray absorptiometry, were positively and significantly associated with body weight and height (correlation coefficients ranged from 0.39-0.81, $P<0.001$). Using multiple- regression analyses among four sex-ethnical groups, weight and height explained 64% and 67% of variance of the total appendicular muscle mass in African-American women and Caucasian women, respectively. Corresponding values for men were 63% in African-American and 39% in Caucasian men. Contrasting to the study of Janssen et al (384), associations between body size and muscle mass were linear in this study.

A further study by Kuh et al (389) in 2775 men and women of the age of 53 years showed that from the first fifth of weight to the last, handgrip strength increased from 44 to 50 kg (P for trend <0.001). Corresponding trend for fifths of body height was 44 to 51 kg (P for trend <0.001). Similarly, Arden et al (47) reported significant correlation between body size and handgrip strength in 706 postmenopausal women. Correlation coefficients of handgrip strength and leg extensor strength with body weight were r = 0.18 and r = 0.13, respectively. Similar correlations were found for height (r = 0.24 and r = 0.22, respectively). These results suggest the importance of body size in predicting muscle strength. It may be explained by the fact that bigger subjects require greater skeletal muscle mass for body movements.

2.7.3.5. Genetics

A classic twin study by Arden et al (47) found a modest genetic component for muscle strength. Handgrip strength and leg extensor power were measured in 277 pairs of MZ and 126 pairs of DZ female twins aged 45-70 years. After adjustment for age, height and

weight, estimated heritability of handgrip strength and leg extensor strength were 0.36 (P = 0.03) and 0.60 (P = 0.01), respectively. These results are consistent with another twin study (386), in which handgrip strength of 152 twin pairs (77MZ and 75 DZ pairs) was measured repeatedly at mean ages of 63 and 73 years. The heritability of handgrip strength was found to be 35%. Similar study on 127 pairs of MZ twins and 130 pairs of DZ twins (387), reported a heritability of 65% for handgrip strength after adjustment for weight, height, age, and various anthropometric measures of fatness, muscle mass, and frame size. Animal studies have given similar results (388).

There is evidence indicating a racial difference in long life decline in muscle mass. Aloia et al (382) in a study, stated above (section 2.7.3.1), reported that from the age of 20 to 70 years white women lost 22% of the total body potassium (TBK), while blacks lost only 8%. Correspondingly, correlation of TBK and age was much greater in whites than in blacks, which was not statistically significant (r =-0.42, $P < 0.001$, and r =-0.14, $P = 0.18$, respectively).

2.7.3.6. Early life exposures and muscle mass (Programming)

Adult muscle mass might be affected by environmental influences acting during intrauterine and early postnatal life, a phenomenon called "programming", mentioned in previous sections. Association between size at birth and later muscle function has been found in several studies and provided evidence for the effect of early nutrition on muscle mass in later life. In this regard, Kuh et al (389) in a study of 2775 men and women of the age of 53 reported that handgrip strength was positively and significantly associated with birthweight, and weight at the age of 7 (P<0.001) in men and women. Men and women within the highest fifth of the distribution of birth weight had 10 percent greater grip strength than did their counterparts in the lowest fifth of the distribution. Furthermore, an extra kilogram of birth weight was associated with a 3.05-kg difference in grip strength for men and a 2.00-kg difference for women. After adjustment for body size at the age of seven and current age, the association between birth weight and handgrip strength remained strongly significant. Likewise, a study of 895 men and women showed that lower weight at age one was significantly associated with lower handgrip strength in adults (390). Phillips (391) reported lower muscle mass, estimated by urinary creatinine secretion, in adults

whose birthweight was lower than 2.5kg in comparison with those with birthweight of 3.5kg or more. These results indicate the effect of early nutrition and growth on predicting adult muscle mass and muscle strength.

2.7.3.7. Summary

Age-related decline of muscle mass and strength is a multifactorial condition, in which many factors are involved; some are inevitable effects of age and others maybe modifiable. Understanding of the underlying factors of sarcopaenia, as the main intrinsic factor of the risk of falling, is important for preventive purposes. Undernutrition, reduced physical activity, hormonal and genetical factors are among the major determinants. What is neglected in the published literature is evidence of relationship between usual diet and muscle strength. Clarifying the relationship between muscle performance and dietary intakes will put light to the effect of nutrition on the risk of fall.

2.8. Summary of the literature review

The literature review presented was concerned about nutritional aspects of bone health and muscle function. It may be considered as having two sections. The first of these presented the metabolism and physiology of bone and discussed how bone health may be affected by various nutritional and non-nutritional factors in the elderly and how it may be monitored by biomarkers. The second section examined the literature and evidence of the effects of various factors on muscle function in the elderly.

2.8.1. Diet and bone health

Studies that have examined the association between diet and bone health produced inconsistent results. Most studies have focused on a single, or a few nutrients, measured in isolation and mostly conflicting results have been reported. The results of such studies are difficult to interpret because of strong correlations and interactions among various nutrients and foods. Although focusing on individual foods or nutrients might provide important data on relations between nutritional variables and bone health, it does not address questions about the multidimensional nature of diet and complex effects of dietary factors on bone. Additionally, strong intercorrelation among some nutrients (such as calcium, protein and vitamin D) makes it difficult to examine their effects separately. Furthermore, they may interact not only among themselves, but also with other genetic and environmental factors. This may be among the main reason for discrepancies in the results of various studies in this regard. An alternative approach might be considering nutritional intakes in the context of the entire diet. Analysing of overall dietary patterns may be more comprehensive regarding to collinearity between different foods and nutrients. Using this approach, complicated interactions among nutrients and other non-nutritional components are more likely to be taken into account. Furthermore, such an approach is more parallel to the real world, where people eat foods not nutrients. Results of such analyses are much easier to be translated to the public for nutritional recommendations and dietary interventions.

During recent years analysing dietary patterns has received more attention in epidemiological studies. The relation between dietary patterns and some chronic diseases in the elderly such as cancer, coronary heart disease, blood pressure and diabetes mellitus (409-

412) have been reported in numerous recent studies but, to my knowledge, such information on bone health is lacking. Investigation on the effects of dietary patterns on bone health is, therefore, clearly needed.

2.8.2. Non-nutritional variables and bone health

Beside different nutrients, a great number of other factors, which may affect bone health, were also discussed in the presented literature review including sex, age, tobacco smoking, physical activity, weight and weight change. The results of previous studies with respect to the effects of different variables are of particular importance in order to know how these factors may affect bone health and how they interact with each other. These results may have implications for oncoming researches. In this regard, sex seems to act as an effect modifier on the associations between most dietary and non-dietary factors and bone health. The pattern of relationships between bone health and most nutritional and non-nutritional factors were different in men and women. The differences by gender may suggest important hormone related effects, in particular that of sex hormones, which undergo dramatic deterioration in women at menopause but rather gradual changes in men (105).

Age, physical activity and alcohol use are other factors, affecting both bone health and dietary intakes and seem to act as confounding variables with inconsistent reported evidences.

Cigarette smoking may adversely affect bone health. Smokers are less active and have lower calcium intake and more likely to be vitamin D undernourished (235;238;413). Most studies of smoking and bone health (21;155;159;161;226), but not all(220;244), have reported a negative association between cigarette smoking and bone health. In this regard, cross-sectional studies (235;238) showed stronger harmful effect of smoking among men than women without any effect of number of cigarettes smoked. Similar results were obtained from cohort studies (233;234)but with a dose-dependent trend. There are some data suggesting an interaction between female sex hormones and smoking habits (239). These data suggest that smoking may act as an effect modifier for sex hormones in females. However, this premise has not been confirmed by another study on young women (240) and for men it remains to be discovered. Clearly, it must be applicable

within the population under study and must be considered in the study design before going to the final analyses.

The effect of early growth on bone health in adults and elderly is also of particular importance. Growth is the major determinant of the skeletal size, which in turn is related to bone mass (40;41). Skeleton provides an envelope, in which bone tissue must be laid-down. Skeletal size in adults is programmed in early life and thereby exposures in infancy and childhood may affect bone health in the elderly (40). Early growth is well reflected by height and weight since weight in infancy is highly correlated with adult height and to a lesser extend with adult weight, what is known as "tracking". These two, are indicators of body size and therefore the skeletal size.

Summing up, reported information in published literature led me to conclude that the traditional single-nutrient approach is not adequate to address questions about nutritional aspects of bone health. I am suggesting, therefore, that there may be a link between bone health and dietary patterns as seen in other chronic diseases in the elderly, such as coronary heart disease and cancer. Analysis of overall dietary patterns in relation to bone health may provide reliable information to overcome discrepancies seen in current literature of bone health and nutrient intakes.

Relations between dietary patterns and bone health might be confounded by some factors other than nutritional ones. Age, alcohol use, malnutrition, smoking, caffeine consumption, low physical activity are known as risk factors for bone health. Sex and body size may also modify these relationships. Sex differences may represent the effect of sex hormones on bone. In postmenopausal women the skeleton is influenced by a huge deterioration of sex hormones and, therefore, the priority in causal pathway is of hormonal changes rather than dietary variation, therefore, it would be expected that the effect of dietary variables on the skeleton be less strong in women than men. Body size may reflect early nutrition as well as long-term nutritional state in the past. However, it is worth noting that bone health in the elderly represents the cumulative effect of many risk factors, both past and present, and including both genetic and environmental, that have affected bone for many years.

2.8.3. Biochemical bone markers

The literature of monitoring bone metabolism by biomarkers were also reviewed. Based on the presented evidence in this regard, bone mass, bone loss and the risk of fracture can be well predicted by bone markers. In this respect, total alkaline phosphatase (T ALP), as the most commonly used marker to evaluate bone metabolism, was found to be well associated with bone mass and fracture risk (331;333-335). Additionally, compared to the bone-specific isoenzyme, T ALP was found to be of a great value, particularly in epidemiological studies, as in established osteoporosis there my be no preference of B ALP over T ALP (339;340). Furthermore, it has been shown that the main isoform responsible for the variations in T ALP associated with age, sex and the menopause is B ALP (414). Therefore, association between dietary intakes and T ALP can be confidently considered as a landmark of the effect of diet on bone. However, it is essential to rule out hepatic diseases when T ALP is used in epidemiologic studies.

2.8.4. Muscle performance and diet

Evidence regarding to the factors associated with the risk of fall in the elderly indicate that there may be a link between diet and muscle performance, which is a major determinant of falling risk. Although a few studies on nutrition and muscle performance showed weaker muscles in undernourished individuals, whether usual diet can affect muscle performance is still unknown. Studies, in which dietary intakes have been correlated with the muscle performance, mainly focused on the effect of vitamin D and produced inconsistent results.

3. Subjects and methods

All analyses undertaken in this thesis are based on the data of the National Diet and Nutrition Survey (NDNS) of people aged 65 years or over in mainland Britain (59). This chapter describes the sample selection and other procedures of NDNS. It will also present the methods of the studies, which will be described in chapters four though seven.

Selection of the database of NDNS was because of; 1) providing high quality of dietary information on a population-based survey (4-day weighted dietary record, coding about 3500 food items), 2) being representative of people most at risk for osteoporotic fractures in the UK, 3) providing detailed information on important socio-economic and lifestyle characteristics which are important for food intake behaviours and bone health as well as different blood analytes. The main limitation of the data for the study on diet and bone health is that it provides a snapshot of the population and from a cross-sectional analysis of the data, causality can not be inferred. Another limitation may be that we had no control on the obtaining the information and therefore some information are lacking in this survey such as menopausal state, lactation information, puberty and peak bone mass, these are because the survey was not primarily purposed for bone health. Limitations of the study will be discussed later on the last chapter.

The survey was commissioned by the Ministry of Agriculture, Fisheries and Food and the Department of Health, and undertaken by Social and Community Planning Research (SCPR), University College London Department of Epidemiology and Public Health and the Dunn Nutrition Unit, Cambridge. The primary data from the survey is lodged with the University of Essex Archive (The Data Archive, University of Essex, Wivenhoe Park, Colchester, Essex CO4 3SQ, UK, telephone +44 (0)1206 872001, fax +44 (0)1206 872003), from which the permission for the analysis of the data has been obtained.

Fieldwork was carried out from October 1994 to September 1995 and included assessment of food and nutrient intakes and a wide range of nutrient and other blood status indices as well as anthropometric and demographic measures. The sample was nationally representative and included two groups of subjects: one from free-living individuals and the other from those living in institutions.

Main objectives of the survey adopted from the official report (505) were:

(1) Assess food consumption, nutrient intakes and nutritional status of individuals and groups, as a basis for Government policies;

(2) Establish reference ranges of nutrient intakes and status markers;

(3) Monitor the population's diet for adequacy and variety;

(4) Monitor progress towards dietary targets (Department of Health, 1991);

(5) Examine evidence for relationships between diet, morbidity and mortality;

(6) Estimate intakes of additives and contaminants for risk assessment;

(7) Assess dental and oral health status, and its relation to diet and nutrition

The survey had the following components(59):

- A detailed interview to provide general information about dietary habits and back-ground information about lifestyle, health status, medication and socio- economic characteristics. Questionnaires to assess mood and depression and memory were also included;
- A weighed dietary record of food and drink consumed over four consecutive days;
- A seven-day record of the number of bowel movements;
- Physical measurements (height, weight, demi-span, mid upper arm circumference, waist and hip circumferences, hand grip strength and blood pressure);
- A blood sample (if written, witnessed consent is obtained) analysed for a range of haematological and other blood indices of nutritional status;
- A single urine sample for analysis of urinary sodium, potassium and creatine;
- An interview to provide information on oral health and an oral health examination.

Sample selection and characteristics of the sample are presented here.

3.1. Subjects

3.1.1. The free-living sample

To be representative of people aged 65 and over in Great Britain, free-living subjects were recruited by sex and age groups. Men and women in age groups of 65-74 years, 75-84 years and over 85 years were recruited by equal number of 230 in each group, except that of men over 85years, which was calculated to be 100 persons.

The sample of free-living subjects was recruited using a multi-stage random probability design. Using Postal Address File (PAF), a total of 80 postal sectors were randomly selected, with probability proportional to the number of postal delivery points.

Postal sectors were stratified according to region and the 1991 Census data for social class. From each selected postcode sector, 375 addresses were randomly selected and were asked to provide information about the sex and age of all household members by filling a sift form, sent by post. The overall initial response rate to form was 85% (45% of the sift forms retuned by post and 40% returned by interviewers during the follow-up of non-responders). From eligible addresses, a sample of individuals was recruited using the probabilities required to produce the target sample size (1250), with only one person being selected per household. From 2,172 eligible addresses, 1,632 adults co-operated with the main survey questionnaire (75%), of whom 1275 subjects (59%) provided a full 4-days weighted dietary records and 986 (45%) provided blood samples. Non-responders were visited by interviewers for collecting the same data as responders.

3.1.2. The institution sample

The sample of people in institutions was drawn by selecting nursing and residential homes for older people in the same postal sectors as the free-living sample. From 195 eligible institutions, 162 institutions agreed to participate (83%), from which 454 residents were sampled with no more than three persons to be selected from any institution. 428 subjects co-operated with the main survey questionnaire (94%), of whom 412 (91%) persons provided a full 4-days dietary records and 290 residents provided blood samples. Selection of the institution sample was performed without considering the age group distribution, and equal numbers of men and women were selected.

Sampling procedure was tested by a feasibility study conducted in February and March 1994 (415). The methodology of subject selection and procedures are described in full in the official survey report (59).

Basic characteristics of the dietary sample (those who provided a 4-day dietary record) are presented in Table 3.1. Data of these subjects have been used in all analyses presented in this thesis.

Table 3. 1- Basic characteristics of dietary sample by sex and domicile (NDNS).

	Men		Women		Total
	Free-living	Institution	Free-living	Institution	
N	632	204	643	208	1687
Age*	73 (6)	82 (8)	75 (7)	78 (7)	76 (8)
Weight*	76.5 (12.4)	66.2 (13.1)	65.0 (12.3)	56.3(11.0)	68.2(14.0)
Height*	169.8 (6.9)	164.1 (8.1)	156.2 (6.4)	150.5 (8.4)	161.2(9.9)
BMI*	26.5 (3.7)	25.1 (4.8)	26.7 (4.7)	24.7 (4.5)	26.4 (4.4)
Proxy information	3%	34%	3%	37%	11%
Smokers	17%	18%	14%	6%	15%
Ex-smokers	60%	54%	30%	22%	43%
Nrver-smoker	23%	27%	56%	72%	42%
Long illness	71%	76%	675	79%	71%
Risk of malnutrition‡					
High	5%	12%	7%	13%	7%
Medium	5%	9%	8%	6%	7%
Low	91%	78%	85%	81%	86%
GGT above normal range	7%	14%	5%	6%	6%
Energy intake (MJ)*	8.0 (1.9)	8.1 (1.9)	5.9 (1.4)	6.9 (1.5)	6.9 (1.9)
EI/BMR**<1.2	41%	28%	59%	26%	46%
ALP IU/l*	87 (33)	110 (72)	94 (79)	104 (55)	94 (62)
Ethnicity					
White	98.6%	100%	99%	98.3%	98.8%
Others	1.1%	0.0%	1.0%	0.3%	0.9%
Social class					
I	-	-	7.8%	-	7.5%
II	25.3%	-	26.8%	-	26.5%
III	58.4%	-	45.1%	-	45.5%
IV	9.1%	-	15.0%	-	14.8%
V	4.5%	-	2.9%	-	3.0%
Armed forces	-	-	1.5%	-	1.5%

* Mean (SD), only subjects with reliable measures were considered

**Energy intake (MJ)/Basal Metabolic Rate

† Based on activity index

‡ Based on MAG tool

3.2. Procedures

3.2.1. Dietary assessment

Dietary assessment was based on a four-days recording questionnaire, validated by the feasibility study. Free-living subjects were trained to weigh (using Soehnle Quantratronic digital food scale) and asked to record a full description of all foods and drinks eaten at home and outside the home over four consecutive days, including second helpings and leftovers. The fieldworkers verified the records with the participants (or carers), checking for information quality.

In institutions, served foods and drinks were weighed by interviewer, who visited the participants every day of diet recording and were aimed to weigh at least one breakfast, one lunch and one evening meal during the recording period. Subjects or their carers were asked to keep a record of all foods and drinks eaten each day. Dietary records were coded by interviewers using a code list of 3500 foods and drinks. Composite foods were split to their constituents. Dietary records were coded to 107 food groups. Daily nutrient intakes for each participant were calculated by linking the quantities of each food consumed with the nutrient databank, which was compiled and maintained by MAFF (Ministry of Agriculture, Fisheries and Food). Records of weighed dietary intake were obtained for 1275 free-living people and 412 people in institutions.

3.2.2. Blood analytes

A blood sample was taken by the nurse from 986 free-living and 290 those living in institutions after an overnight fast at the subject's home or institution. Biochemical analyses were undertaken at the Dunn Nutrition Laboratory in Cambridge using standard techniques. The vitamin status index and other biochemical assay procedures are described in detail in the original report (59). Briefly, vitamins A, E and the carotenoids were measured by high-pressure liquid chromatography, vitamin C was measured using a Roche Cobas Bio centrifugal analyser with fluorescence attachment. Calcium, phosphate and alkaline phosphatase were measured by a Roche Kit Cobas Bio assay. All samples were heparinised and kept at -40°C and -80°C until being assayed at the laboratory, with similar duration of storage. Quality control and calibration of procedures are described in full detail elsewhere (59). Measurement of plasma alkaline phosphatase was possible for 1143 respondents and for the specific assay method used in this survey 35-110 IU/L is

respondents and for the specific assay method used in this survey 35-110 IU/L is considered to be normal.

3.2.3. Anthropometrics and grip strength

Body weight was measured by trained nurses using Soehnle Ouantratronic digital personal scales to the nearest 100 grams on a hard level surface. The participant was asked to remove shoes, heavy outer garments, heavy jewellery and loose change and keys. Height was also measured at the same time with a portable, digital telescopic stadiometer to the nearest millimetre, while the participant's head was in the Frankfort plane.

Handgrip strength was measured for each hand, using a handgrip dynamometer designed by Queen's Medical Centre, Nottingham. Participant was seated with the forearm resting on a table, and the elbow at 90 degrees. Subject was asked to squeezed the handgrip dynamometer to maximum force for three seconds whilst was encouraged verbally by the nurse. After demonstration and one sub-maximal trial, which was not recorded, two trials were given on each hand and handgrip strength was recorded to the nearest 100 grams for each trial. The strongest record for each subject was used in the analysis.

Mid-upper arm was measured on the left arm where possible, at the midpoint of the distance between olecranon process (tip of the elbow) and the acromiom process, using a tape with an insertion buckle at the end. Participant was asked to stand straight and the measurement was recorded to the nearest even millimetre, while arm was naked and was hung loosely at the side with the elbow at 90 degrees and the lower arm across the body.

Reliability of each measurement was recorded for each subject by recording any special circumstances, which may have affected the reliability of the measurement. All analyses presented in this thesis are based on information of subjects with reliable measures.

3.2.4. Other procedures

Ethical approval was given by each NHS local research ethics committees for each of the postcode sectors involved. Written informed consent obtained from all participants or their proxies after fully explanation of the procedures.

In order to allow for seasonal variations, fieldwork was distributed through out the year over four waves: October-December 1994, January-March 1995, April-June 1995 and July-September 1995. In each wave 20 postal sectors were selected throughout Great Britain. The numbers of selected households were equal in each wave and the wave samples were nationally representative.

Fieldwork was performed by trained interviewers and nurses, who had been trained by Social and Community Planning Research (SCPR). All survey participants were visited by an interviewer who administered a detailed questionnaire related to diet, life style and health.

A short memory questionnaire was also administered in order to detect mental impairment and the need for proxy information. Based on the interviewer's subjective assessment, information of 3% of free-living subjects and that of 34% of institutionalised subjects was provided by proxy information givers.

Smoking, physical activity, alcohol use, medication, and self-assessment health status were evaluated by the main questionnaire administered by the trained interviewer. Subjects were also asked whether they had any longstanding illness; and whether they had been admitted to hospital in the last 12 months.

Sample selection was performed by strictly random procedures, with no exclusion criteria. Even subjects, who were confused, were included by using proxy information givers.

However, subjects, who fully co-operated with the interviewer were different from others, who failed to fully co-operate with interviewers in terms of sex and age profiles and marital status. In both free-living sample and the institution sample, respondents were more likely to be male, younger (by an average of 1.2 years), married and living not alone, than non-respondents. They were also more likely to be from social class I and II (see Table 2 in Appendix 1). However, data was weighted, where necessary, according to the co-operation rate across subgroups for region, sex, age and whether living in private house or in institutions, in order to avoid biases due to sampling procedures and response rate differences.

The sample was similar to the 1991census data for population of 65 years and over, in terms of sex and age profiles, region and whether living alone, indicating that the sample was representative of the older population in the UK (see Table 1in Appendix 1).

Summing up, 836 men and 851 women aged 65 and over were randomly selected through their Postcode Address across the United Kingdom, of whom 1275 persons were free living subjects and 412 persons were selected from residential and nursing homes for elderly people in the same postal sectors of free-living subjects. Each subject provided a 4-day weighed dietary record and from which intakes of 107 food groups were derived, as well as providing blood and urine samples and completing a detailed questionnaire including life style variables and health status. The characteristics of the sample is presented in Table 3.1.

3.3. Methods

Principal component analysis was used to define dietary patterns and the subjects' scores on each of the identified patterns were used in further analyses as described below:

- Relationships between dietary patterns and other characteristics of the sample, which may have influenced bone health, was investigated and used to characterise dietary patterns.
- The effect of these dietary patterns on plasma alkaline phosphatase activity as a marker of bone health was investigated, after controlling for other bone affecting factors by using multiple regression analysis.
- Using stratified analysis, the effect of body size on the relationship between diet and bone health was investigated.
- Relationships between dietary patterns and handgrip strength, as a determinant of the risk of fall, were examined.

Details of different procedures and methods are presented in relevant sections.

3.3.1. Statistical analysis

Principal component analysis is used to define dietary patterns and to calculate dietary scores for each person (see next chapter for the details of the procedure). Seven identified dietary patterns are characterised according to their correlations with different food

groups. Calculated dietary scores are used in further analyses as proxies of dietary patterns. Associations between these dietary scores and different demographic and lifestyle variables are tested using different procedures such as parametric correlation (i.e. Pearson correlation coefficient for normally distributed data), nonparametric correlation (i.e. Spearman's correlation coefficient for skewed and categorical data) and Analysis of variance (ANOVA). Associations or differences in the presented analyses are shown as being significant at the 95% ($P<0.05$), 99% ($P<0.01$) or 99.9% ($P<0.001$) confidence levels. The terms "significant" and "statistically significant" are used interchangeably to indicate these significant associations or differences. Where presented results are shown or described as being "non-significant", indicates that $P>0.05$.

ANOVA is used to evaluate the associations between dietary patterns and different lifestyle variables which were shown in bivariate analyses to be related with food intakes, including smoking, physical activity, risk of malnutrition, age and domicile. A full factorial model of ANOVA is used for each dietary pattern, separately and it is conducted for men and women in institution and free-living groups, independently. All analyses were controlled for energy intake. To compare the differences in dietary scores among different subgroups for domicile, smoking, age groups, long illness and physical activity, a number of different procedures is used (e.g. one way ANOVA, independent sample t-test and conservative tests such as Student- Newman-Kelus, Tukey and Bonferi).

To evaluate the associations between dietary patterns with ALP, as a marker of bone health, and handgrip strength as a marker of muscle performance stepwise multiple regression analysis is used and subjects with extreme values (defined as standardised residual >3SD) are excluded from the analysis. Using "Casewise diagnostic procedure" for each analysis, existing of such subjects in the data is checked. In order to assess the effect of "healthy diet", as the main dietary pattern related to ALP, on the risk of having high level of plasma ALP (defined by median), a model of logistic regression analysis is used for men and women, independently and controlled for different confounders and energy intakes.

Analyses are conducted on the data of dietary sample (those who fully co-operated with the survey and provided a full 4-day weighted dietary record). The results for the whole

sample, domicile and gender subgroups are presented to compare associations within each subgroup. All analyses are controlled for extraordinary situations which may have affected the results, such as subjects who suffering from morbid diseases i.e. cancer, kidney and endocrine diseases or situations, which may directly affect ALP or handgrip strength. For ALP, analyses are controlled for hepatic diseases (diagnosed by γ–glutamyl transferase >50 IU/L for men and > 32 IU/L for women) and kidney dysfunction (diagnosed by plasma BUN/Creatinin ratio> 15) by excluding those who suspected to have these diseases. Furthermore, women who were using medicines for obstetric and gynecologic treatments are also excluded. For handgrip strength, those who were using musculoskeletal medicines are excluded from the analyses. The probability of underreporting and its effect on the result is also considered and is presented in the Appendix 1. Statistical analyses were performed using the SPSS for Windows version 11.0.

4. Dietary patterns in the elderly in the UK

In this chapter dietary patterns identified by principal component analysis, will be characterized with respect to the food groups and nutrient contents. The first study aim of characterising eating behaviours among the elderly population in the UK will be addressed in this chapter.

4.1. Principal component analysis

Principal component analysis was used to summarise the diet of the population under study and to identify the dietary behaviour of the elderly population in the UK. Principal component analysis is a method of factor analysis that aims to simplify the correlations between numerous interrelated variables (417). This method is used in order to identify underlying dimensions of dietary behaviour and to summarise correlation patterns among a set of dietary variables by extracting a relatively small number of components, which are called dietary patterns in this thesis. These patterns are indicating how different foods are consumed together and coming to the diet. Extracted components in this approach are then defined by their correlations with the nutritional variables used in the analysis, which here were the average amounts of daily food intakes. Each person will be attributed a score on each of the identified patterns. This approach identifies patterns of eating habits as they exist.

In order to maximise the precision of factoring process we attempted to reduce the number of variables entered the analysis, and thereby, reduce the complexity of data, by combining similar foods into certain groups and collapsing food groups of the original data into smaller number of food groups. Grouping scheme was based on the similarity of nutrient profiles or culinary usage among the foods and greater emphasis placed on similarity of foods with respect to those nutrients that are thought to affect bone metabolism. For example: all dairy products were combined together regardless of their fat contents and all breads were combined together regardless of their fibre contents. Some individual food items were preserved because it was inappropriate to incorporate them in to a certain food group such as eggs, soup, sugar and ice cream. Bacon and ham, burgers and kebabs, lamb and pork dishes, beef and veal and sausages were combined into the red meat group. None of initial foods or food groups was omitted from the list. In this manner, initial food

groups (107 foods and food groups) were collapsed into 44 defined food groups. Our grouping scheme was somewhat similar to that used in other studies (410;412;418). Grouping scheme was not based on a primary hypothesis. It was closely similar to that used before in the Dietary and Nutritional Survey of British Adults (418) with almost similar dietary information, and was very similar to that used by other studies (412;430), who showed the reliability of dietary patterns defined by factor analysis using food consumption data collected through an FFQ(430) . The group names and mean daily intakes of these food groups are presented in Table.4.1.

4.1.1. Procedure of principal component analysis

The average daily intakes (grams) of foods from each of the 44 defined food groups for 1687 persons were used in the analysis. For initial analysis no limitation was placed on the number of factors to be generated. Then the eigenvalues (>1), the turning point on Scree plot (Figure 4.1) and the difference between percentage of variance explained by each successive components were used to determine the number of components to be used for the final analysis. Scree-plot in the Figure 4.1 illustrates the eigenvalues for factors, generated initially by factor analysis. Eigenvalue of a factor is the sum of square of factor loadings of all variables (here are food groups) with that factor and reflects the amount of test variance explained by the factor (the total variance for each test is 100% or unity).

Considering these eigenvalues we selected the first seven components because changes in eigenvalues between successive components after the seventh component were not substantial. Consequently, the turning point of Scree plot was used to determine the number of component to be selected. Increasing the number of considered components did not increase the percentage of variance, considerably. Obviously, a great number of dietary patterns would be needed to account for a high percent of the variation in food intakes and such a large number of patterns would be difficult to interpret.

Table 4. 1-Mean daily intake of 44 foods or food groups assessed by 4-day weighed dietary record by 1687 participants in NDNS in the UK.

Food or food groups	Mean (g/d)	Std. Deviation
Pasta, Rice, Pizza and cereals	13.9	26.8
Breads	89.8	46.5
Breakfast cereals	47.4	70.8
Biscuits	16.5	18.8
Buns, cakes and pastries	30.8	31.0
Fruit pies	5.88	15.36
Puddings	46.0	56.3
Milk and other dairy products (yoghurt, Fromage frais, dairy desert)	268.5	167.5
Cream (including imitation)	2.7	7.1
Cottage and other cheese	10.5	14.1
Eggs	17.8	22.8
Butter	6.8	10.9
Margarine	2.1	6.02
Oils	0.1	1.0
Low fat spread	9.0	11.5
Red meat	63.3	47.6
Chicken and turkey	16.9	27.9
Liver and dishes, liver pate and liver sausage	2.1	8.6
Meat pies, pastries and other Meat products	22.8	31.8
Fish and shellfish	30.0	30.0
Carrots, Peas and green beans	27.3	27.2
Baked beans	8.0	18.3
Salad and other vegetables	36.0	38.7
Tomatoes	18.4	24.9
Leafy green vegetables (incl broccoli)	15.8	21.5
Fried or roast potatoes products (crisps and chips)	28.9	33.8
Not fried Potato products	70.4	52.5
Apples, pears, Citrus fruits, Bananas (not canned)	52.4	67.2
Canned fruits	16.2	32.8
Other fruits	17.4	36.8
Sugar	18.9	25.8
Preserves	8.4	12.1
Sweet spreads and Confectionery products	4.1	16.6
Drinks – fruit juice	20.6	52.8
Soft drinks	66.8	137.6
Liqueurs Spirits	4.3	17.4
Wine	9.1	40.3
Fortified wine	2.5	13.3
Beers, lagers, Cider and Perry	76.8	280.6
Low alcohol and alcohol free beer, lager, cider and perry	2.6	24.8
Coffee ,Herbal tea and tea (made up weight)	772.4	341.6
Soups	34.7	64.4
Ice cream	9.2	16.8
Nuts and seeds (including fruit and nut mixes)	.54	3.9

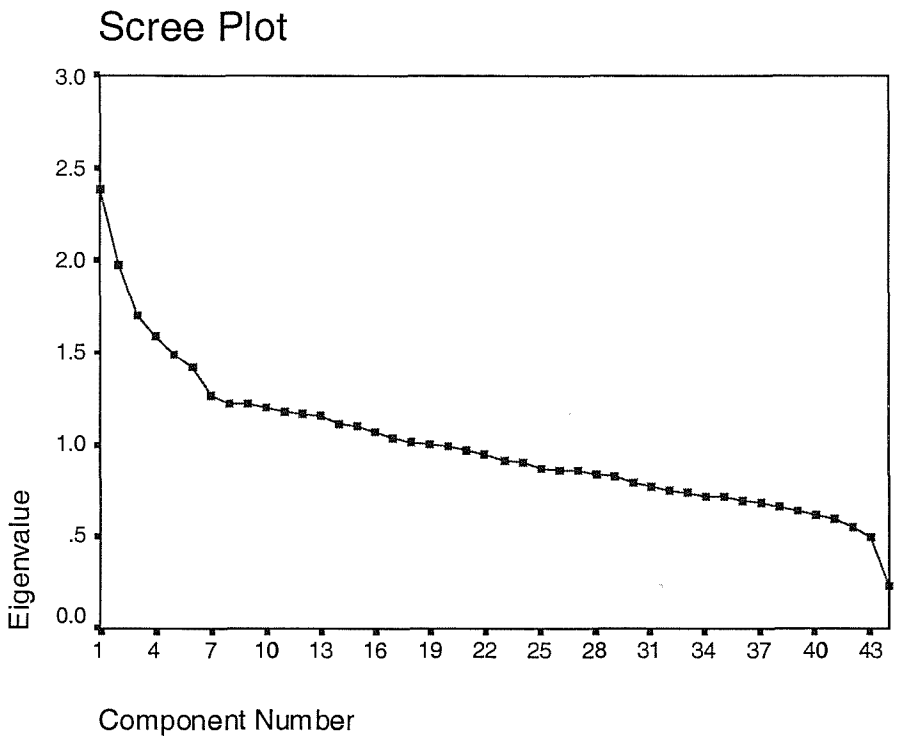


Figure 4. 1- The Scree test of principal component analysis of 44 food groups among 1687 participants in NDNS in the UK.

The next step replicated the preliminary analysis, considering seven components determined in the previous step. In order to maximise the relationships between the food groups and increase the interpretability of components, the Varimax method of rotation was used, which redistributed the explained variance across the components without affecting the total percentage of variance explained. The first seven components explained a total of 27.4% of the total variation in the food intake between individuals, from which the largest amount was related to the first component (5.5%). Subsequent components accounted for progressively smaller amounts of the variation.

4.1.2. Dietary scores

Factor scores for each dietary pattern were computed for each subject by summing up the products of multiplication of factor loading of each food group with the standardised values of the reported amounts of consumed foods (using regression method for saving scores as variables in SPSS for Windows version 11). Dietary scores were normally distributed with the mean value of zero across the total sample. They were statistically dif-

ferent (all correlation coefficients were 0.00, P =1.0). For each subject it was possible to have high scores on each of the dietary patterns. These scores were used in subsequent analyses.

4.1.3. Characteristics of dietary patterns

In attempting to define each dietary component or eating pattern, correlations of the different food groups on each of the seven rotated components were considered. In order to highlight the stronger associations between dietary patterns and food groups, factor loadings (the correlations between each food groups and related component or dietary pattern) of less than 0.25 were omitted. Note that this cut off point was selected arbitrarily, because considering all correlations between components and food groups would produce a long list of food groups for each component and would be difficult to interpret. Please note that cut-off points were used only for presenting the data, but not for inclusion in any analysis. By entering dietary scores into an analytical model, all their correlations with food groups are taken to account. Negative loadings indicate the less likelihood of consumption of concerned foods for subjects who have higher score on the corresponding component. These correlations were also omitted if were more than –0.25. Correlations of each dietary pattern to food groups are presented in Tables 4.2-4.8 and relations between dietary patterns and nutrient intakes are also shown in Table 4.9. Characteristics of dietary patterns were as below:

4.1.3.1. Component one

This pattern had a strong positive correlation with fresh fruits and vegetable consumption. It also was correlated with the intake of fish, shellfish, chicken and turkey. Negative association to the intake of sugar and puddings was seen and therefore, this component can be considered as a "Healthy diet". It is important to note that this dietary pattern is closely consistent with the healthy dietary guidelines in the UK (448). Consumption of variety of foods, having a plenty of fruits and vegetables in the diet, eating plenty of foods rich in starch and fibre, avoiding the consumption of too much sugary foods and drinks and not eating too much from foods that contain a lot of fat are the key points of the guidelines which are compatible with the characteristics of the "healthy diet" identified by principal component analysis in this study. Because of contrasting correlations of this eating pattern

with healthy and unhealthy eating behaviours, each subject's score can indicate how one may eat healthy or unhealthy foods.

Table 4. 2- Component 1 (Healthy dietary pattern).

Food group	Factor loading
Apples, pears, Citrus fruits, Bananas (not canned)	0.55
Salad and other vegetables	0.53
Other fruits	0.50
Fruit juice	0.39
Tomatoes	0.39
Wine	0.38
Cottage and other cheese	0.37
Chicken and turkey	0.33
Nuts and seeds (including fruit and nut mixes)	0.26
Fish and shellfish	0.25
Puddings	-0.26
Sugar	-0.42

% Total variance = 5.5

Conceptually, higher scores on this dietary pattern indicate stronger trend toward healthy foods and stronger trend away from unhealthy foods, and vice versa. It was strongly associated with the intakes of vitamin C, magnesium, potassium and dietary fibre (Table 4.9).

4.1.3.2. Component two

This component was strongly correlated to the intake of breads, coffee and tea. Other food groups that were covered by this dietary pattern were potato and red meat, including bacon, ham, Beef, veal, Lamb, Pork and Sausages. It may be considered as a "traditional meat-trend diet". The second dietary pattern showed the strongest correlation with protein, energy, zinc, copper and vitamin D intake among all patterns. A wide range of minerals such as calcium, Mg, phosphorus and potassium was also provided by this dietary pattern (Table 4.9).

Table 4. 3- Component 2 (Traditional meat trend diet)

.Food group	Factor loading
Breads	0.72
Coffee, Herbal tea and tea (made up weight)	0.56
Low fat spread	0.37
Preserves	0.33
Red meat	0.29
Cottage and other cheese	0.28
Fried or roast potatoes products (crisps and chips)	0.25
Tomatoes	0.26
Soft drinks	-0.28

% Total variance = 4.6

4.1.3.3. Component three

The third dietary pattern was more related to canned fruits and sweet foods, which may indicates less culinary practice and therefore, less likely to eat fresh foods. This dietary pattern was correlated with the consumption of dairy products and the intake of protein, calcium, potassium and phosphorus. High sugar consumption and energy intake was seen in people with high scores on this dietary pattern. This pattern may be referred to as "sugary food and dairy diet".

Table 4. 4- Component 3 (Sugary-dairy diet).

Food group	Factor loading
Puddings	0.59
Canned fruits	0.46
Milk and other dairy products (yoghurt, fromage frais, dairy desert)	0.37
Buns, cakes and pastries	0.37
Ice cream	0.34
Sugar	0.33
Cream (including imitation)	0.32
Soft drinks	0.31
Breakfast cereals	0.31
Preserves	0.30

% Total variance = 3.9

4.1.3.4. Component four

This dietary pattern represents people who were interested in alcoholic beverages and meat products. It was strongly associated with the alcohol intake. People with high scores on this dietary pattern were more likely to eat meat products and eggs but less likely to eat dairy products. All correlation coefficients between this dietary pattern and nutrient intakes were less than 0.25 except for alcohol consumption and energy intake. It had a weak negative correlation to the intake of calcium (-0.07, $P<0.001$). All alcoholic drinks except fortified wine showed strong loadings on this dietary pattern and thus, it may be considered as an "alcohol-trend diet".

4.1.3.5. Component five

By this dietary pattern people have higher intake of green vegetables but lower intakes of animal products, and, therefore, can be referred to as a "vegetarian-trend diet". Higher fibre, vitamin A, C and potassium but lower protein, calcium and phosphorus intakes were related to the scores of this dietary pattern.

Table 4. 5- Component 4 (Alcoholic-trend diet).

Food group	Factor loading
Liqueurs Spirits	0.55
Beers, lagers, Cider and Perry	0.47
Meat pies, pastries and other Meat products	0.37
Eggs	0.34
Nuts and seeds (including fruit and nut mixes)	0.30
Wine	0.29
Milk and other dairy products (yoghurt, Fromage frais, dairy desert)	-0.26

% Total variance =3.7

Table 4. 6- Component 5 (Vegetarian-trend diet).

Food group	Factor loading
Not fried Potato products	0.71
Leafy green vegetables (Including broccoli)	0.58
Carrots, Peas and green beans	0.51
Margarine	0.30

% Total variance =3.4

4.1.3.6. Component six and seven

The sixth and seventh dietary patterns were less strongly related to the intake of different nutrients and energy, although explained a considerable amounts of variance in food intakes in the population (a total of 6.2%). The sixth dietary pattern was strongly associated with the intake of low fat spreads. It was also negatively associated with the butter intake. It was correlated with the intakes of vitamin D, calcium and phosphorus (correlation coefficients were -0.16, -0.04, and –0.06, respectively, $P<0.01$). The seventh was strongly related to soups, margarine and breakfast cereals. It was negatively associated with wine consumption.

Dietary energy intake was strongly correlated with the second, third and fourth dietary patterns (Table 4.9).

Table 4. 7- Component 6

Food group	Factor loading
Low fat spread	0.77
Butter	-0.71

% Total variance =3.3

Table 4. 8- Component 7

Food group	Factor loading
Soups	0.50
Margarine	0.43
Breakfast cereals	0.31
Baked beans	0.25
Liver and dishes, liver pate and liver sausage	-0.29
Fortified wine	-0.36

% Total variance =2.9

Table 4. 9- Pearson's correlation coefficients between dietary scores and nutrient intakes [a].

	Dietary patterns						
	Healthy	Traditional	Sugary-dairy	Alcohol-trend	Veg-trend	Comp6	Comp 7
Vitamin A					0.25		
Vitamin C	0.62				0.28		
Vitamin E	0.25	0.38				0.43	
Vitamin D		0.25					
Zn		0.43	0.27		0.25		
Cu	0.29	0.32					
Fe	0.37	0.37	0.26				
Mg	0.45	0.35					
Calcium		0.24	0.53				
Phosphorus	0.33	0.38	0.39				
Potassium	0.51	0.36	0.28		0.29		
Protein	0.31	0.52	0.26				
Energy		0.53	0.51	0.35			
Alcohol				0.66			
Fibre	0.48	0.38			0.33		
Fat		0.53	0.42	0.32			

* All correlations are significant at the level of $P<0.01$.

[a] Correlation less than 0.25 are omitted.

4.2. Life style variables

4.2.1. Physical activity assessment

Assessment of physical activity was based on an interviewer-administered questionnaire, by which participants were asked to report their habitual activities on sports, walking, housework and gardening. Three walking activity levels were defined according to the duration: walking lasting 1-2 minutes, 5-10 minutes, and for more than 20 minutes continuously. The frequencies of these walking activities were ranked by nine levels: two

times a day or more, once a day, 5-6 times a week, 3-4 times a week, twice a week, once a week, once a fortnight, once a month and less often. Subjects were asked about their walking pace, which was categorized as slow, steady or average, fairly fast or fast (4mph). Sport activities were assessed on the following sports: Cycling/riding exercise bike, exercises for fitness, exercises as part of physiotherapy, dancing, swimming, running/jogging, badminton or tennis, golf, yoga, bowls, rambling and others. The frequencies of these activities were ranked by six levels: every day, 4-6 times a week, 2-3 times a week, once a week, once a fortnight and less often.

Light and heavy gardening, light and heavy house works, carrying loads, climbing stairs and occupational activities were also assessed in a similar manner. No information about the intensity of activities was collected and so no energy cost could be calculated.

Using comparative energy costs of different activities according Durnin and Passmore (419), separate summary indices were created for each subject on each activity and then an over all index of physical activity was created.

A score was calculated from the frequency and the comparative energy cost of different sport and domestic activities, according to Durnin and Passmore (419). According to their comparative energy costs, using standardized tables (419), activities were divided into three categories; the "light activities" require relatively lower energy output and were included: golf, bowling, exercises as part of physiotherapy, yoga and rambling light housework and light gardening (average energy expenditure of 0.76 MJ/h). The "moderate activities" included: badminton or tennis, Cycling/ riding exercise bike, swimming, dancing, exercises for fitness, carrying loads, climbing stairs, heavy housework and heavy gardening (average energy expenditure 1.26 MJ/h). Vigorous activities included running or jogging (average energy expenditure 1.76 MJ/h).The lowest frequency of the light sports was used as the referent.

A walking score was also calculated from the frequency and time spend on walking, using the lowest category as the referent.

In summary, a composite index summing various activities, with respect to their frequency and comparative energy costs, was computed and expressed as physical activity

index (PAI). Frequency distribution of PAI for the entire dietary sample is presented in Figure 4.2.

According to the sex and domicile-specific thirds of physical activity scores, subjects were categorised as low active (mean of activity score = 80, n = 543), moderately active (mean of activity score = 167, n = 529), or very active individuals (mean of activity score = 278, n = 535). As stated above, this approach of classification of activities was based on standard tables (419). Similar approach of creating activity index was used in previous studies (420) but no validation was performed against physiological measures of physical activity. However, classification of activities was similar to that used in the Health Survey for England (421).

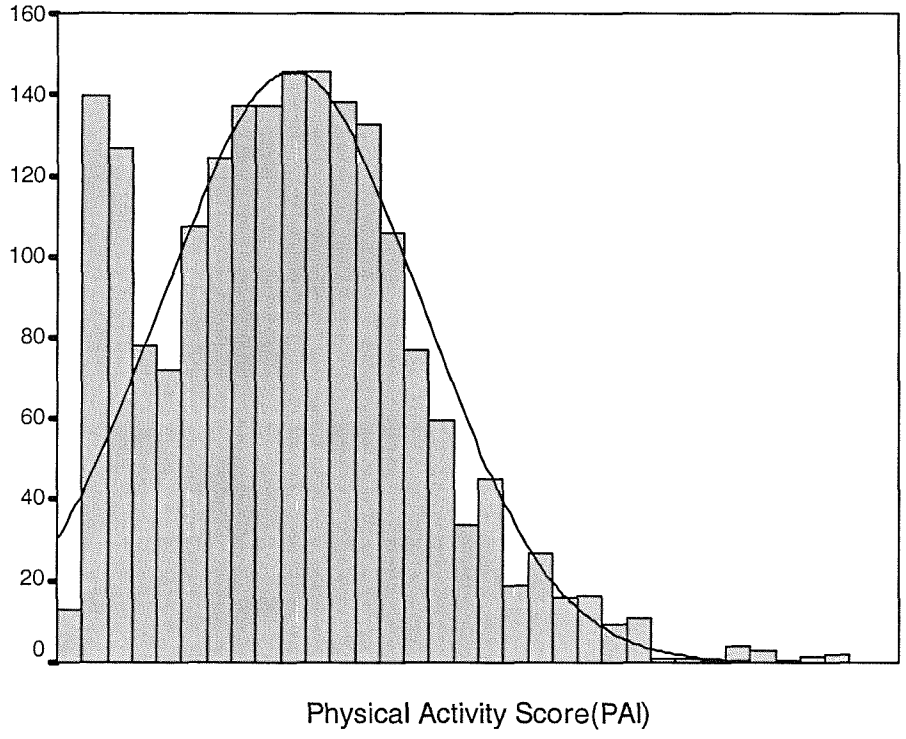


Figure 4. 2- Frequency distribution of total physical activity score among men and women (NDNS).

However, it was different from other methods in that no information about intensity and duration of most activities was available and so no energy cost can be calculated.

Presently, there is no widely accepted objective measure of physical activity that serves as "gold standard" and, therefore, for validation of the approach, it must be inferred indirectly from correlations of physical activity with other physiological measures. In this re-

spect, correlations to BMI, energy intakes and High-density lipoprotein cholesterol (HDL-C) might be used, as several studies showed positive association of physical activity with energy intake and HDL-C and negative association with BMI (422-424).

In attempting to validate the PAI in our population, we compared the mean values of BMI, HDL-C and energy intakes among activity groups, using a model of one-way analysis of variance (ANOVA). Activity groups were significantly different in terms of all three examined variables (Table 4.10), therefore, our approach in evaluating of physical activity may be of value.

Table 4. 10- Validation of physical activity index (PAI); one way ANOVA and descriptive of BMI, HDL-C and Energy intake by activity groups.

	F ratio, P value	**Mean**	**95%CI**	
BMI	5.5 (2,1320) P= 0.004			
Low active		26.78	26.23	27.34
Moderately active		26.68	26.18	27.18
Very active		25.67	25.34	26.01
HDL-C	8.6 (2,1065) P= 0.000			
Low active		1.19	1.14	1.25
Moderately active		1.25	1.21	1.30
Very active		1.34	1.29	1.38
Energy intake	9.1 (2,1601) P= 0.000			
Low active		6.59	6.41	6.76
Moderately active		7.02	6.87	7.17
Very active		7.22	6.98	7.46

4.2.2. Smoking assessment

Subjects were asked to report their past and current smoking habits for the smoking of cigarette, cigar and pipe. Based on the collected data they were categorised into three groups: current smokers (n= 291), never smokers (n = 896) and past smokers (or ex-smokers) (n =865). The amount of current tobacco consumption was calculated for smokers and converted to cigarette equivalent units, according to Todd GF (425). For past smokers the total amount of lifetime cigarette smoking was also calculated as Pack-year, which means smoking of one pack of 20 cigarettes per day, for one year. Because of the lack of information on the frequency of pipe smoking, only cigarette and cigar smoking were considered in calculation of current cigarette consumption in this group. Among per-

sons who provided a 4-day dietary records 16% (n = 249) were current smokers, 39% (n = 720) had quitted cigarette smoking, and 45% (n = 712) had never been regular smokers.

4.3. Malnutrition

As has been discussed in previous chapter, general protein undernutrition and weight loss are referred to as risk factors for bone health. Using a modified form of MAG (Malnutrition Advisory Group) screening tool (96), we created an index of the risk of malnutrition for each subject. In this approach a combination of weight loss and BMI would define the risk of undernutrition (protein-energy malnutrition) (PEM). Assessment of weight loss in this survey was based on the interviewer administered questionnaire, by which subjects were asked if they had lost weight over the last six months. Those who lost weight were asked about the amount of weight they thought they had lost and their answers were categorized into one of four categories: less than three pounds (<1.4Kg), three pounds to less than half a stone (1.4 -3.2 Kg), half a stone to less than one stone (3.2-6.4Kg), and more than one stone (>6.4Kg). These categories were adopted according to MAG tool as the percentage of weight loss as below: the first and the second groups were considered as weight loss of <5% of body weight (weight loss of less than 1.4kg to 3.2kg), the third category was considered as losers of 5-10% of body weight (weight loss 3.2-6.4kg) and the last category was considered as losers of >10% of body weight (weight loss >6.4).

4.3.1. Definition of malnutrition risk

According to MAG tool and information obtained in the survey, each subject fell into one of three groups: high, medium or low risk for undernutrition. Groups were defined as:

- High risk: BMI <18.5 plus any amount of weight loss, **Or** BMI 18.5-20 and weight loss ≥3.2. **Or** BMI >20 plus weight loss more >6.4Kg.
- Medium risk: BMI, 18.5-20 with no weight loss or weight loss <3.2.**Or** BMI>20 and weight loss 3.2-6.4.
- Low risk: BMI>20 and no weight loss or weight loss < 5%.

Intention to lose weight was not considered in this scheme because of lack of data. This may lead to a misclassification of those who intended to lose weight by falsely categorising them as high or medium risk of malnutrition. Low risk people may be categorised as medium or high and medium risk people may be categorised as high risk of malnutrition.

If this is true, it may lead to bias in associations between malnutrition risk and any other variables as well as ALP by showing no relation or attenuate the association between malnutrition risk and plasma ALP.

The percentages of malnutrition among men and women are presented in Table 4.11. A total of 6.9% of the whole sample were at high risk of malnutrition with a higher percentage among women in comparison to men (6.2% vs. 7.7%).

Table 4. 11- Distribution of malnutrition risk among men and women (NDNS) evaluated by MAG tool.

	Malnutrition risk		
	Low	Medium	High
	N(%)	N(%)	N(%)
Free-living			
Males	518 (90.6)	26(4.5)	28 (4.9)
Female	474 (85.3)	45 (8.1)	37 (6.7)
Total	992(87.9%)	71(6.3%)	65 (5.8%)
Institution			
Males	94(77.0)	13(10.7)	15 (12.3)
Females	96(81.4)	7(5.9)	15(12.7)
Total	190 (79.2%)	20 (8.3%)	30 (12.5%)
Age group			
Men			
65-74	251(92.3)	10(3.7)	11(4.0)
75-84	255(87.3)	17(5.8)	20(6.8)
85+	106(81.5)	12(9.2)	12(9.2)
Women			
65-74	204(86.8)	17(7.2)	14(6.0)
75-84	194(82.6)	19(8.1)	22(9.4)
85+	16(7.8)	16(7.8)	172(84.3)
Long illness			
Yes	667(85.3%)	63 (8.1%)	52 (6.2%)
No	324(94.2%)	8 (2.3%)	12 (3.5%)
Total	1182 (86.4%)	91(6.7%)	95(6.9%)

4.4. Long standing illness

Each subject was asked about their health and any long standing illness, disability or infirmity, which affect the participants over a period of time. For those reported long standing illness, the kind of disease was also recorded. In total 71% of men and 67% of women had reported to be affected by long illness. Among those reported to have an illness for a long time, 2% had cancer and 7% had suffered from diabetes. People with longstanding illness were at higher risk of malnutrition than others without longstanding illness (6.2% vs. 3.5%) (Table 4.11).

Figure 4.3 and 4.4 present the distribution of different lifestyle variables (activity and smoking groups, high/medium and low under nutrition risk groups, and long illness) among dietary sample (those who completed 4-d dietary records) by sex and domicile.

4.5. Dietary patterns and other characteristics of the sample

Different statistical methods were used to demonstrate the relationships between identified dietary patterns and other variables, which may affect food preference, bone health or both. Factor scores of individuals on each of the dietary patterns, obtained by principal component analysis were used as proxy of eating patterns. Relationships between dietary scores and other characteristics of the sample including age, domicile, smoking habits, physical activity and health status (whether being unwell for a long time or not) were investigated for men and women, separately.

Spearman's correlation coefficients were calculated between dietary scores, age and physical activity index. The results are presented in Table 4.12.

Age Age was strongly and negatively related to the scores on healthy and traditional diets (patterns one and two) in both sexes, but it was positively associated with the "sugary food and dairy diet", characterised by high intakes of cakes, pastries, canned fruits and dairy products (Table 4.4). In spite of strong positive correlation coefficient between age and sugary food diet and high correlation coefficient between this dietary pattern and energy intake, age was negatively associated with energy intake. It was not surprising, because most of other dietary patterns were negatively related to age. It may indicate lower food intake in the elderly and higher proportion of energy intake from carbohydrates in older subjects in comparison with younger ones. In general, age was negatively associated with the scores on most dietary patterns. This appears to be due to lower dietary intakes in older subjects, partly because of lower physical activity, and thus, lower energy intake. In this regard, age was found to be negatively and strongly correlated with activity in both sexes (r_s = -0.42 and –0.50 in men and women, respectively, P<0.001) and was negatively associated with energy intake in men (r_s = -0.14, P<0.001). However, corresponding association in women was not statistically significant (r_s = -0.02, P = 0.47).

Using partial correlation coefficients, association between dietary scores and age was controlled for energy intake and physical activity (Table 4.13). Most dietary patterns were negatively associated with age, suggesting lower dietary intakes by age. In both sexes strong negative association was found between healthy dietary pattern and age as well as strong positive association between age and the third dietary pattern (sugary food-dairy diet) after controlling for energy intake and physical activity. It may suggest that older subjects are consuming more from sugary and dairy foods and less from healthy foods and this trend could not be explained only by variation in energy intake or physical activity. This showed that negative associations between some dietary patterns and age are partly due to food preference. Preference of sugary-dairy diet by older individuals might be due to lower cooking skills. If an individual has no skills in cooking then they have restricted options in what they can eat, as there may be a tendency to rely upon processed and canned foods. They are also less likely to use raw ingredients in their cooking such as fruits but in particular vegetables and healthy foods. Similarly, without cooking skills, control over what is purchased and eaten may also be compromised, as there will be little opportunity to try and diversify the diet to include healthier foods. Furthermore, cooking and cooking skills are part of the wider frame work of food choice involving menu planning, budgeting and purchasing (453). In NDNS, it appears that ability of preparing foods is related to dietary scores. When people were divided by their maximum dietary scores, healthy eaters (those with highest scores on healthy diet) were more likely to cook their own food than those who mostly practiced sugary-dairy diet (67.9% vs. 48.2%, χ^2 = 33.72; df =12, P =0.001), suggesting that choosing more from the third dietary pattern and less from the healthy diet in the elderly in the UK may be partly due to less ability of preparing food. Intake of foods also may be limited by sensory perception including taste. Brug et al (454) reported that only those people who liked the taste of fruits and vegetables were eating them, whilst liking them were a major motivation for their consumption. Beside the taste and ability to cook, availability of foods and the ability of an individual to access food and storage facilities, as well as dental problems in elders may affect their food preference.

Table 4. 12-Spearman's correlation coefficients of the dietary scores with physical activity index, and age in men and women (NDNS).

	Healthy	Tradi-tional	Sugary-dairy	Alcohol-trend	Veg-trend	Comp 6	Comp 7
Men (n = 836)							
Age	-0.22	-0.25	0.21	-0.15	-0.01	-0.14	0.01
P value*	0.00	0.00	0.00	0.01	0.16	0.00	0.80
Physical activity	0.35	0.17	-0.19	0.09	0.05	0.09	-0.05
P value*	0.00	0.00	0.00	0.01	0.15	0.01	0.14
Women (n = 851)							
Age	-0.29	-0.11	0.28	-0.05	-0.11	0.09	-0.02
P value*	0.00	0.00	0.00	0.12	0.00	0.01	0.54
Physical activity	0.33	0.11	-0.25	-0.06	0.08	0.03	-0.09
P value*	0.00	0.00	0.00	0.10	0.03	0.40	0.01

*Pvalues are two-tailed

For healthy eating pattern, a negative trend for age groups is seen in Figure 4.5, similarly in men and women, but this pattern was not similar for domicile groups (Figure 4.6).

Institutionalized subjects had similar ranges and quartiles on healthy dietary pattern across various age groups, while, in free-living ones, older groups had lower scores. This negative trend toward healthy diet across age groups was also evident for never smokers and ex-smokers but not for smokers (Figure 4.7). However, as is shown in Figures 4.18 among those from institution sample, healthy dietary score did not varied by different background and lifestyle variables, substantially. It may be due to low variability of food intake in these subjects because of limited food choice in the institutions. Preparing foods by kitchens in institution may have affect the food intake of institutionalised individuals and give them less opportunity to try and diversify the diet to include healthier foods.

Physical activity Physical activity was positively associated with the healthy diet and negatively related to sugary food-dairy diet in men and women, similarly (Table 4.12). The strongest correlation was seen between healthy diet and physical activity in both sexes. Negative association of physical activity to sugary food-dairy diet and strong positive associations to healthy diet may indicate that more active people are more health-conscious with respect to their diet. Controlling for energy intake did not change these relations, substantially.

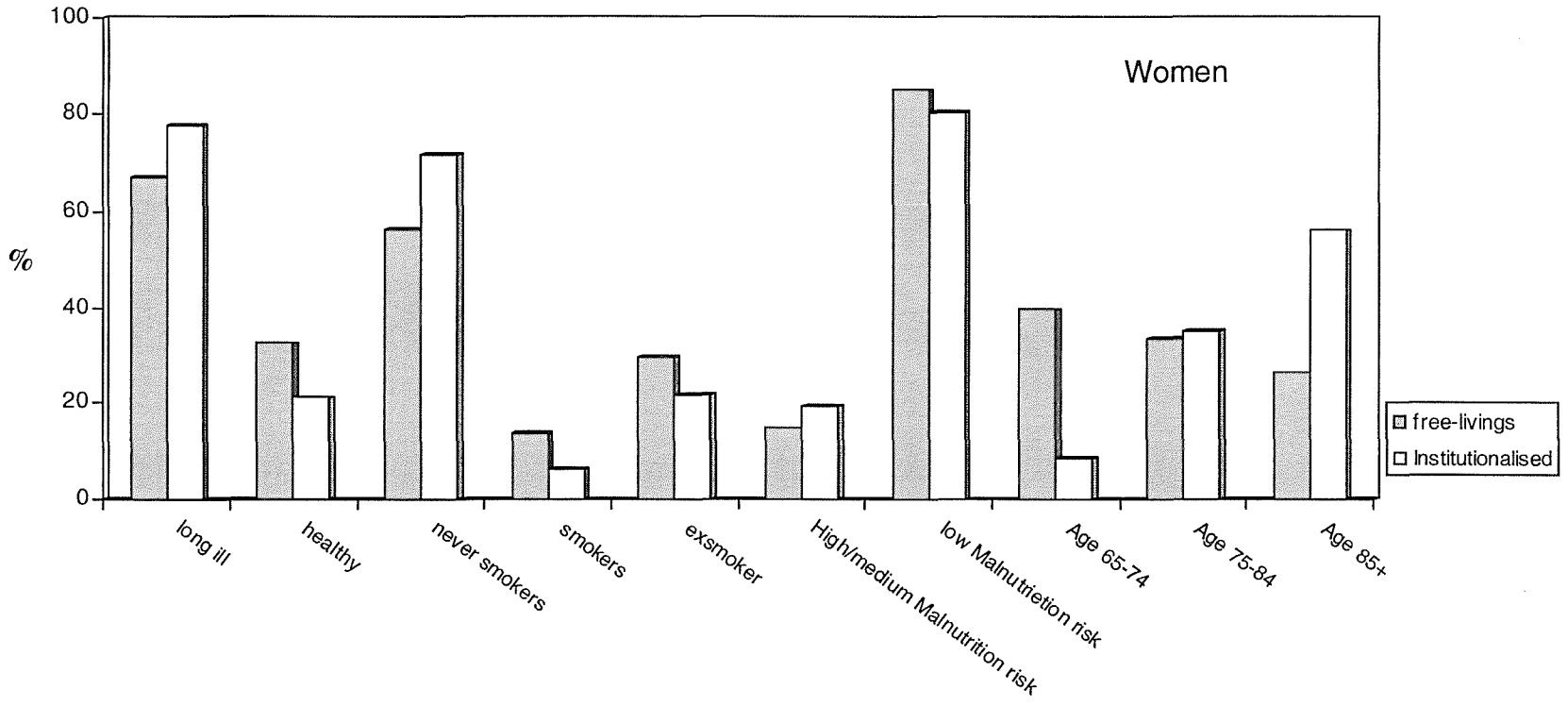


Figure 4. 3- Distribution of background and life style variables in females (NDNS)

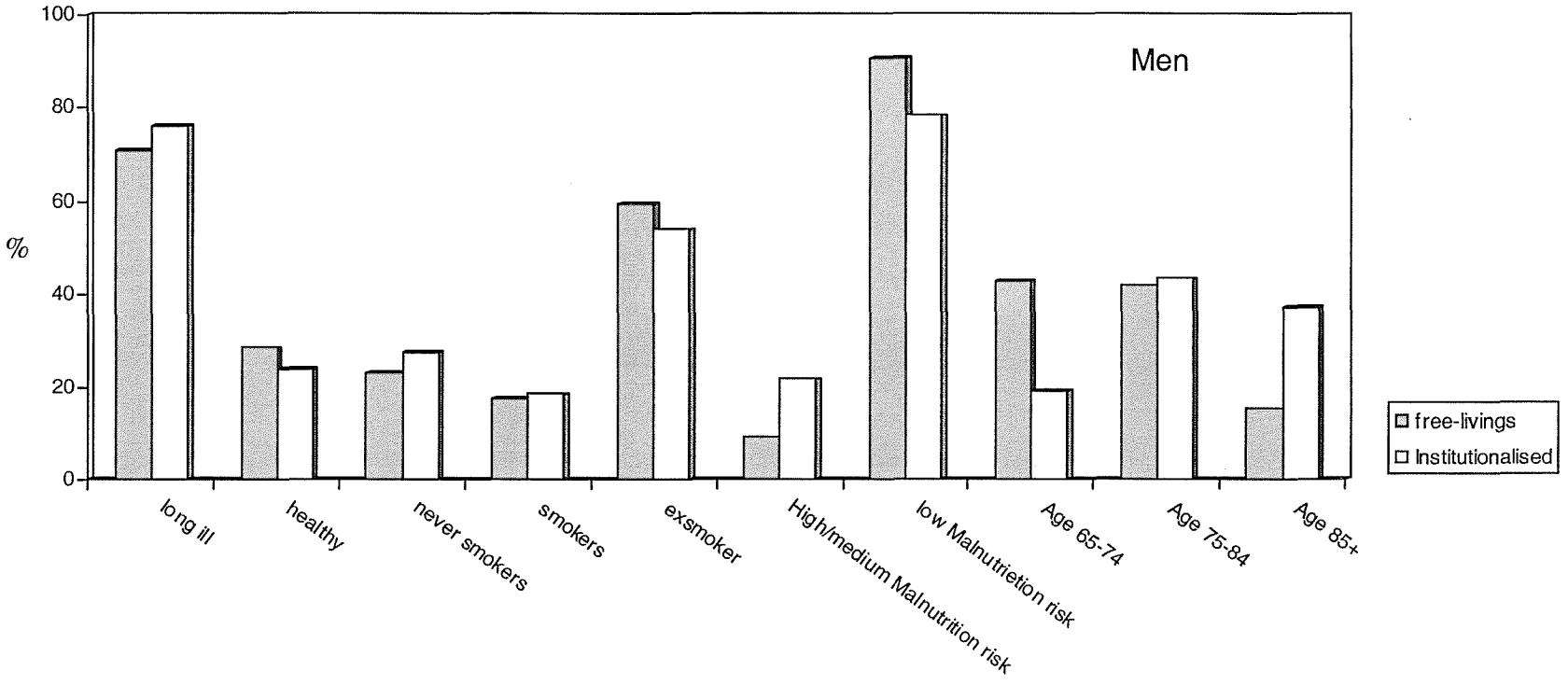


Figure 4. 4- Distribution of background and lifestyle variables in males (NDNS).

Table 4. 13- Partial correlation coefficients between dietary scores with and age, controlled for energy intake, in men and women (NDNS).

	Healthy	Tradi-tional	Sugary-dairy	Alcohol-trend	Veg-trend	Butter	Marga-rine
Men (n = 833)							
Age	-0.18	-0.20	0.31	-0.10	-0.01	-0.14	0.03
*P** value	0.00	0.00	0.00	0.02	0.80	0.00	0.43
Women (n = 848)							
Age	-0.30	-0.12	0.34	-0.07	-0.12	-0.10	-0.01
*P** value	0.00	0.00	0.00	0.05	0.00	0.01	0.86

*Pvalues are two-tailed

Figure 4.8 depicts the effect of activity on distribution of healthy dietary pattern among men and women. Similar patterns were seen among men and women with a positive trend for physical activity, indicating healthier eating habit among more active subjects, although, the ranges of different activity groups are somewhat overlapped. Less active subjects were different from the two other groups (moderate and very active subjects) with respect to healthy diet score and age. They were older (Figure 4.9) with fewer trends toward the healthy diet (Figure 4.8).

Relations between smoking habits and dietary patterns were also investigated. One-way analysis of variance and Student- Newman-Kelus were used to detect differences in the eating patterns with respect to the smoking habits in men and women, separately. The means and 95% confidence intervals of dietary scores are presented in Tables 4.13 and 4.14. Among women, differences between smokers, never-smokers and ex-smokers were significant only for the scores on patterns one, three and four (healthy, sugary food-dairy and alcoholic-trend diets). Among men these differences were significant for patterns 1-5.
In both sexes ex-smokers had significantly higher scores on "healthy diet" compared with never smokers and current smokers.

Smokers from both sexes had significantly lower scores on sugary food-dairy diet and higher scores on alcoholic-trend diet in comparison with the two other smoking groups.

As Table 4.14 shows smoker men had significantly higher scores on the second dietary pattern, characterised by high coffee and tea drink, and lower scores on the fifth dietary pattern, characterised by high intakes of leafy green vegetables, carrots and non-fried potato products.

The two other smoking groups, which together were current non-smokers, did not differ in this regard. However, among women these three groups were not significantly different with respect to these two dietary patterns. Prevalence of smoking was significantly higher in men than in women (19% vs. 16%).

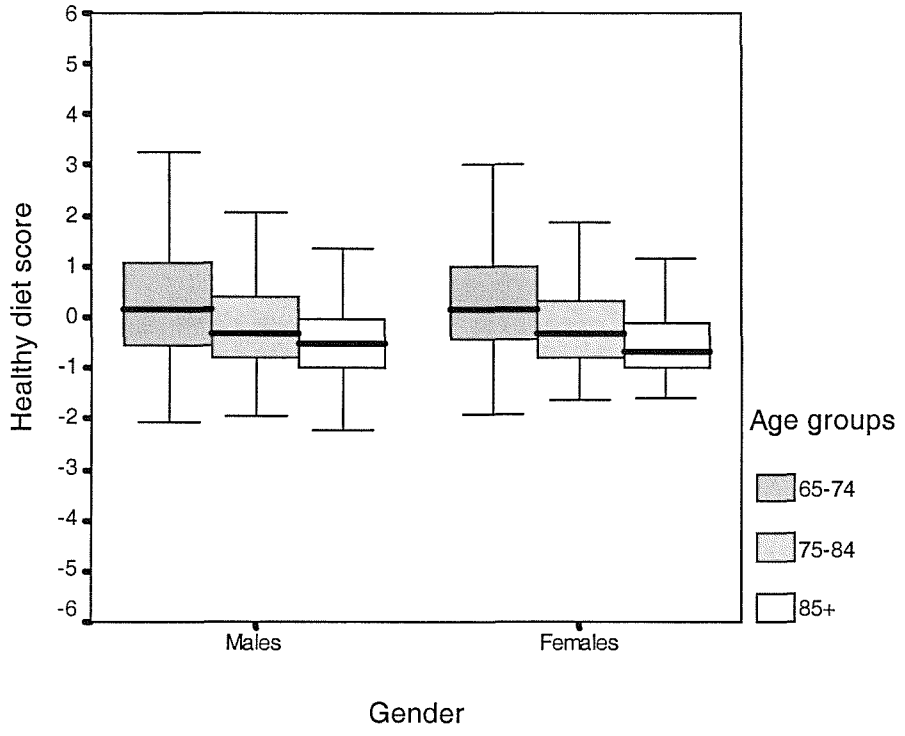


Figure 4. 5- Distribution of healthy diet score by sex and age groups (NDNS).

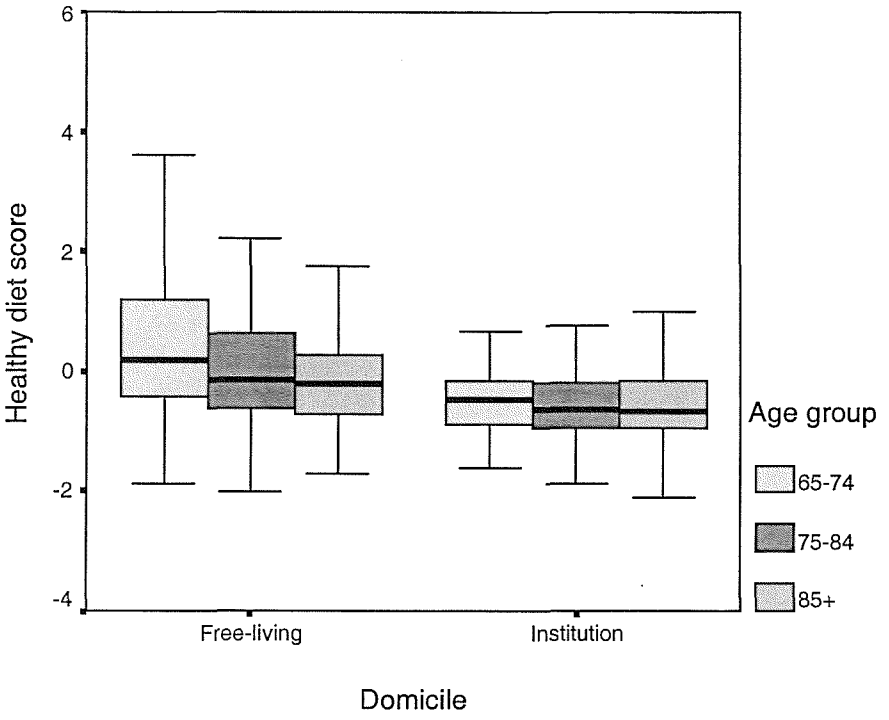


Figure 4. 6-Distribution of healthy diet score by age groups and domicile index (NDNS).

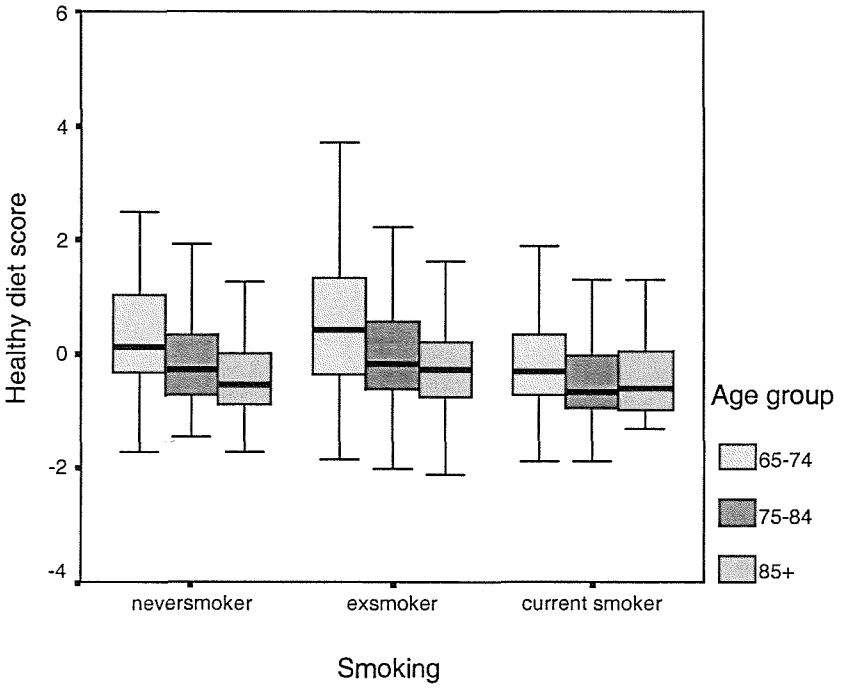


Figure 4. 7-Distribution of healthy diet score across age and smoking groups (NDNS).

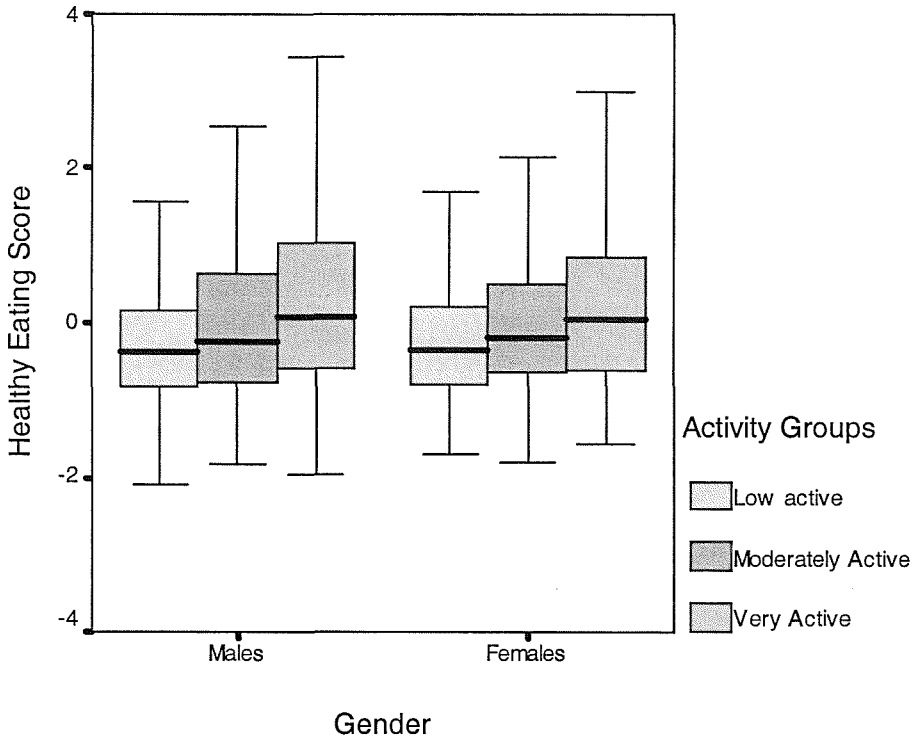


Figure 4. 8-Distribution of healthy diet score by sex and physical activity.

Figure 4.10. shows the distribution of healthy dietary score by smoking groups across genders. Similar pattern for men and women is shown with a little difference between smoking groups. Quartiles and ranges are somewhat overlapped, although the differences are statistically significant (Tables 4.13 and 4.14).

Domicile Free-living subjects were different in terms of dietary intakes from those living in institutions (Tables 4.15 and 4.16). Institutionalised men and women had significantly lower levels of dietary scores on healthy and traditional diets (first and second dietary patterns) and higher levels of scores on sugary and dairy diets. More men in institutions practiced "alcoholic-trend diet" than those in the free-living group.

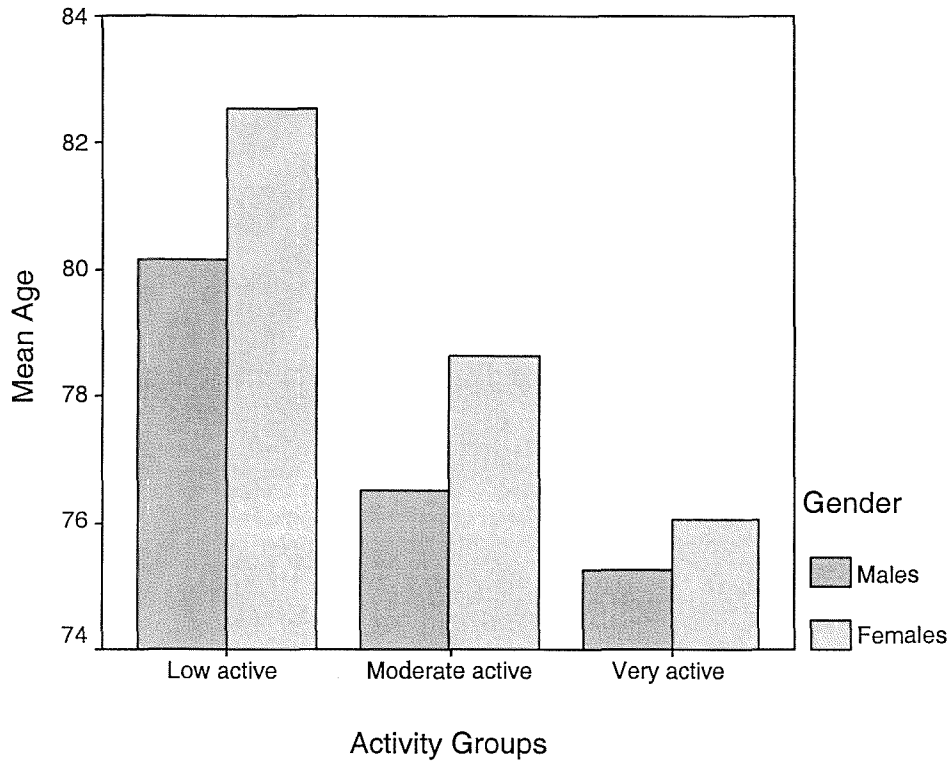


Figure 4. 9- Age distribution by activity levels (NDNS).

Figure 4.11.presents the distribution of "healthy diet" score by sex and domicile index. Similar patterns (with respect to the range, inter-quartiles and median) were shown for men and women in both free-living and institutionalized participants.

Subjects from institution sample were older and less active, compared to the free-living persons (Figures 4.15 and 4.16). More than 87% of subjects living in institutions were non-smoker compared with 84% for free-living persons ($\chi^2 = 12.9_{(df\ =\ 2)}$, $P = 0.00$)(Figure 4.14). Those living in institutions were at higher risk of malnutrition (20%in institutions vs. 12 in free-living people, $\chi^2 = 14.5_{(df\ =\ 2)}$, $P = 0.00$)(Figure 4.15).

Table 4. 14- Distribution of dietary scores by smoking habits among men (n = 832) (NDNS).

Dietary patterns		Mean	95% C I	
Healthy	Never-smoker	0.05	-0.10	0.19
	Ex-smoker	0.12	0.03	0.22
	Smoker	-0.42	-0.59	-0.25
P value	0.00			
Traditional	Never-smoker	0.28	0.13	0.42
	Ex-smoker	0.25	0.15	0.34
	Smoker	0.53	0.36	0.70
P value	0.02			
Sugary-dairy	Never-smoker	0.34	0.20	0.49
	Ex-smoker	0.13	0.04	0.23
	Smoker	-0.20	-0.38	-0.03
P value	0.00			
Alcohol-trend	Never-smoker	0.17	0.00	0.33
	Ex-smoker	0.25	0.15	0.36
	Smoker	0.61	0.42	0.80
P value	0.00			
Veg-trend	Never-smoker	0.27	0.13	0.42
	Ex-smoker	0.12	0.03	0.21
	Smoker	-0.05	-0.22	0.12
P value	0.02			
Pattern 6	Never-smoker	0.14	-0.01	0.29
	Ex-smoker	0.06	-0.03	0.16
	Smoker	0.01	-0.17	0.19
P value	0.53			
Pattern 7	Never-smoker	0.17	0.02	0.33
	Ex-smoker	0.08	-0.02	0.18
	Smoker	0.03	-0.16	0.21
P value	0.44			

Table 4. 15- Distribution of dietary scores by smoking habits among women (n = 849) (NDNS).

Dietary patterns		Mean	95% C I	
Healthy	Never-smoker	-0.08	-0.15	0.00
	Ex-smoker	0.23	0.11	0.34
	Smoker	-0.20	-0.38	-0.02
P value	0.00			
Traditional	Never-smoker	-0.31	-0.38	-0.24
	Ex-smoker	-0.28	-0.39	-0.18
	Smoker	-0.25	-0.41	-0.09
P value	0.75			
Sugary-dairy	Never-smoker	-0.01	-0.08	0.07
	Ex-smoker	-0.20	-0.31	-0.08
	Smoker	-0.60	-0.78	-0.43
P value	0.00			
Alcohol-trend	Never-smoker	-0.32	-0.38	-0.26
	Ex-smoker	-0.34	-0.42	-0.25
	Smoker	0.00	-0.13	0.14
P value	0.00			
Veg-trend	Never-smoker	-0.11	-0.20	-0.03
	Ex-smoker	-0.10	-0.22	0.02
	Smoker	-0.23	-0.41	-0.04
P value	0.48			
Pattern 6	Never-smoker	0.07	-0.14	0.01
	Ex-smoker	-0.05	-0.17	0.06
	Smoker	-0.15	-0.32	0.03
P value	0.66			
Pattern 7	Never-smoker	-0.06	-0.14	0.01
	Ex-smoker	-0.18	-0.29	-0.07
	Smoker	-0.05	-0.21	0.12
P value	0.19			

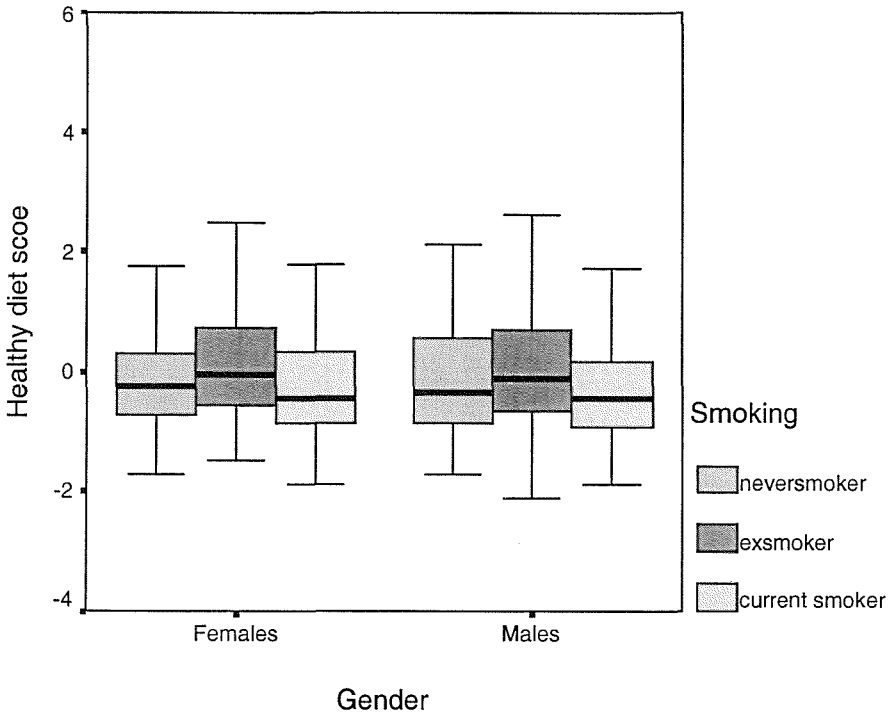


Figure 4. 10- Distribution of healthy diet scores by sex and smoking habits (NDNS). Boxes represent inter-quartile ranges (between 25th and 75th percentiles), horizontal line across the boxes represent the medians and whiskers connect the largest and the smallest values.

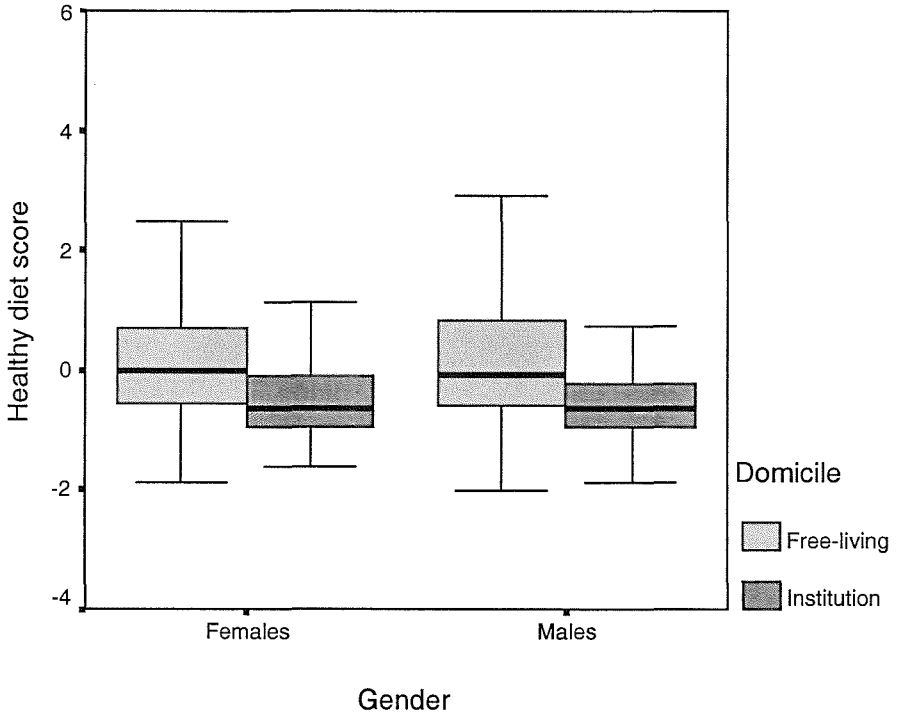


Figure 4. 11- Distribution of healthy dietary score by sex and domicile index (NDNS).

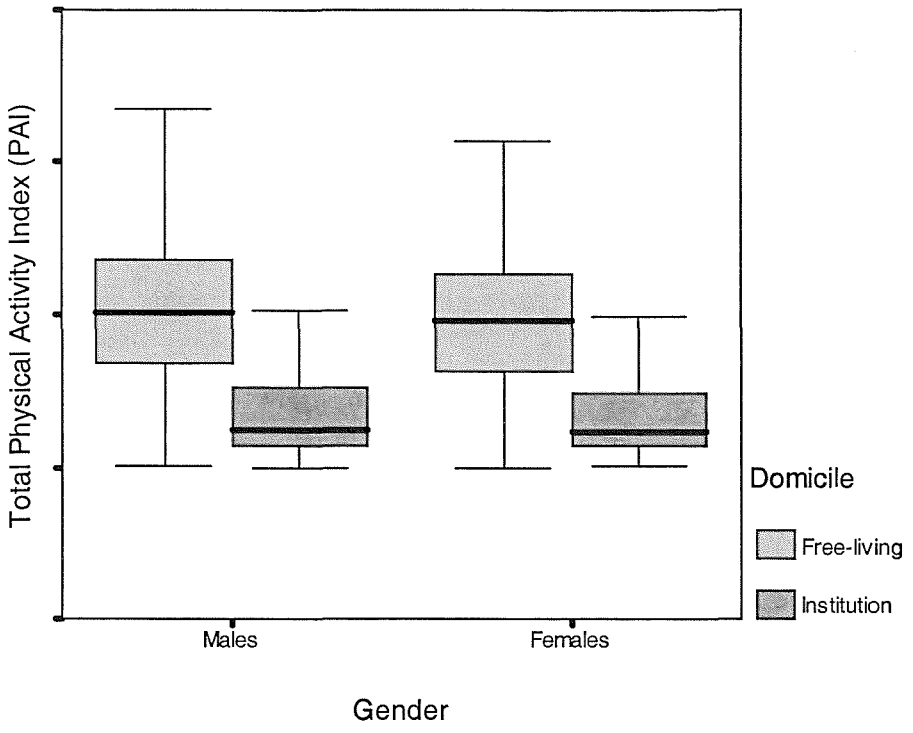


Figure 4. 12- Distribution of activity index by sex and domicile (NDNS).Boxes represent inter-quartile ranges (between 25th and 75th percentiles), horizontal line across the boxes represent the medians and whiskers connect the largest and the smallest values.

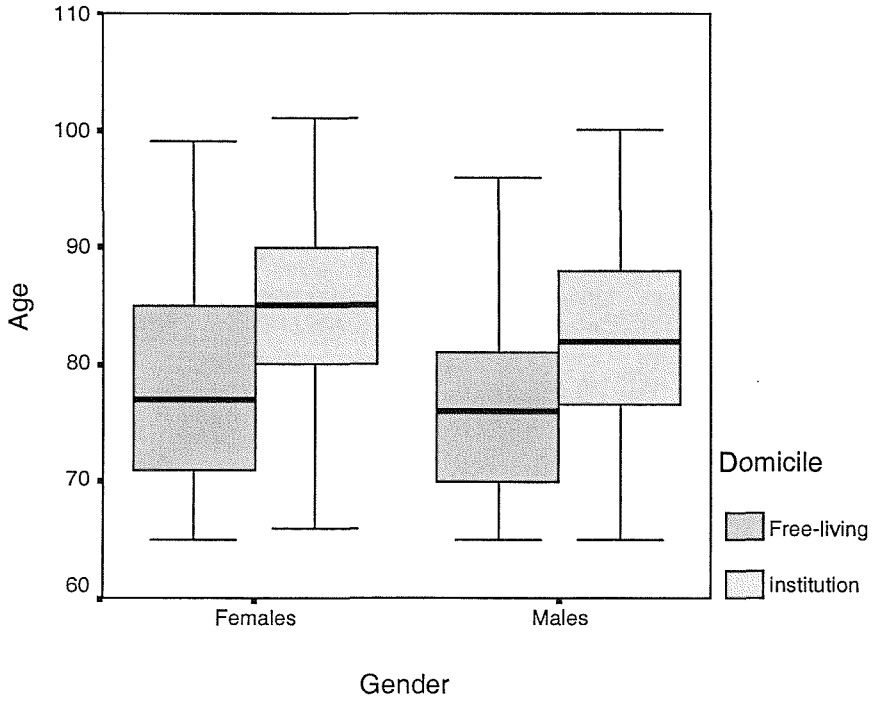


Figure 4. 13- Age distribution by domicile index (NDNS).

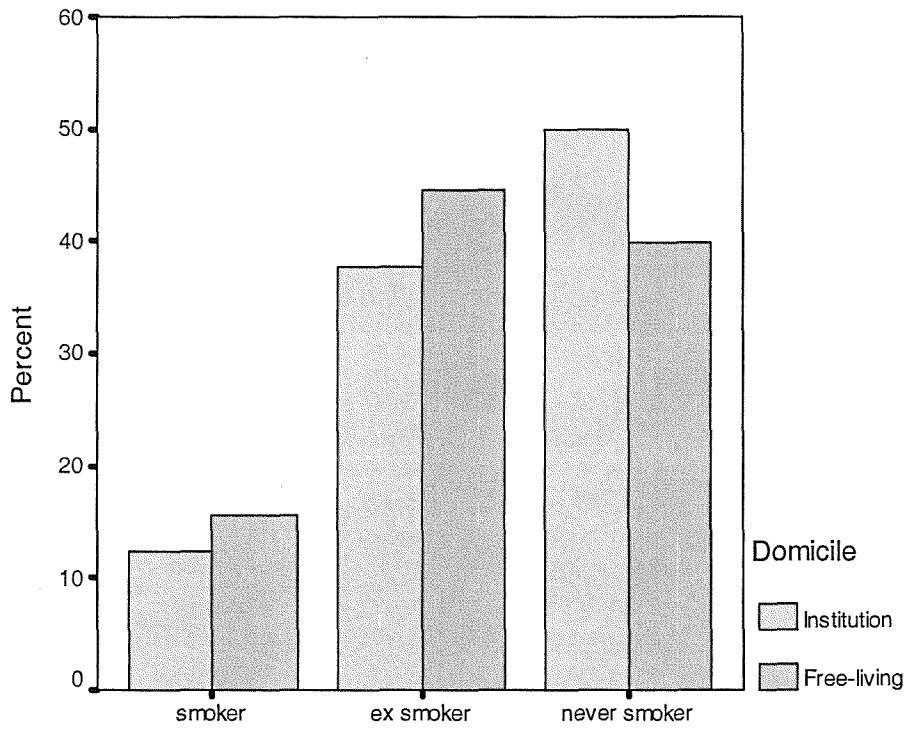


Figure 4. 14- Smoking habits amongst free-living and institutionalised persons (NDNS).

Table 4. 16-Distribution of dietary scores by domicile in men (NDNS).

Dietary patterns	Mean	95% CI	
Healthy			
Free-living	0.19	0.10	0.28
Institution	-0.56	-0.66	-0.47
Traditional			
Free-living	0.41	0.33	0.49
Institution	0.00	-0.15	0.14
Sugary-dairy			
Free-living	-0.13	-0.21	-0.05
Institution	0.91	0.78	1.04
Alcohol-trend			
Free-living	0.33	0.24	0.43
Institution	0.13	0.01	0.25
Veg-trend			
Free-living	0.15	0.07	0.24
Institution	0.06	-0.05	0.18
Pattern 6			
Free-living	0.08	-0.01	0.16
Institution	0.01	-0.13	0.15
Pattern 7			
Free-living	0.01	-0.08	0.10
Institution	0.37	0.23	0.51

Table 4. 17-Distribution of dietary scores by domicile in women (NDNS).

Dietary patterns	Mean	95% CI	
Healthy			
Free-living	0.16	0.09	0.23
Institution	-0.52	-0.60	-0.43
Traditional			
Free-living	-0.27	-0.33	-0.20
Institution	-0.43	-0.55	-0.32
Sugary-dairy			
Free-living	-0.41	-0.47	-0.35
Institution	0.78	0.68	0.88
Alcohol-trend			
Free-living	-0.31	-0.37	-0.26
Institution	-0.18	-0.26	-0.10
Veg-trend			
Free-living	-0.13	-0.21	-0.05
Institution	-0.13	-0.23	-0.02
Pattern 6			
Free-living	-0.09	-0.16	-0.02
Institution	0.03	-0.09	0.14
Pattern 7			
Free-living	-0.17	-0.24	-0.11
Institution	0.15	0.03	0.26

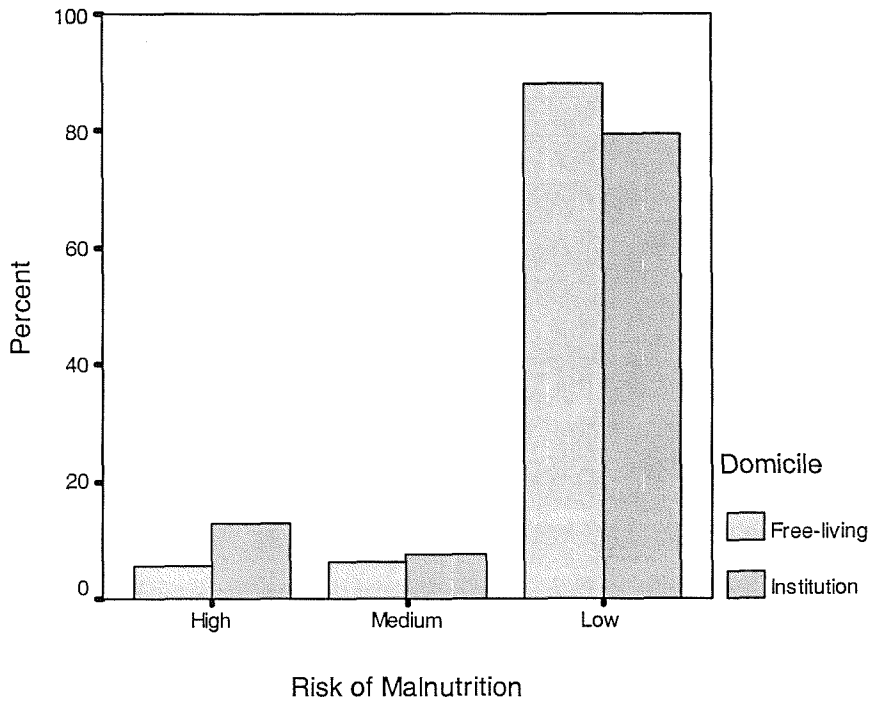


Figure 4. 15-Malnutrition risk and domicile (NDNS).

Malnutrition risk Although men within high-risk group were less likely to eat healthy foods, such difference was not statistically significant in women. As is presented in Table 4.18 a, males with high risk of malnutrition had significantly lower intakes of "healthy foods" and "traditional diet" after adjusting for longstanding illness (remember that people with long illness were at higher risk for malnutrition as presented in Table 4.11). However, using one-way ANOVA, the risk of undernutrition, defined by using MAG tool, was not a significant predictor of most eating patterns in women (Table 4.18 b). However, some care is needed in the use and interpretation of this indicator of malnutrition. Because the intention of subjects to loose weight, which is one of the criteria of this screen tool, was not available in the data used in these analyses. People with intentional weight loss relative to those of unintentional weight loss may be more concerned about their diet (450) and therefore, be at lower risk of malnutrition, while in this study they may be categorised as medium or high risk for malnutrition, because of criteria used in this screening tool, including weight loss and BMI. This may attenuate the differences in healthy diet scores in men and may explain why women within low and high risk groups did not differ in healthy diet score. Intentional weight loss is generally more common in women than in men (449).

Long illness Longstanding illness was not related to the eating behaviours since subjects who reported to be ill for a long time were not statistically significantly different from those who were healthy, either in both sexes or in domicile groups (Figure 4.16 and 4.17).

Table 4. 18 a- Dietary scores by malnutrition risk among men (n=832), ANOVA[1] (NDNS).

Dietary patterns	Malnutrition risk	Mean	95% C I	
Healthy	High	-0.37	-0.79	0.05
	Medium	-0.19	-0.77	0.40
	Low	0.11	0.02	0.21
P value	0.028			
Traditional	High	-0.16	-0.64	0.13
	Medium	-0.01	-0.55	0.53
	Low	0.44	0.35	0.531
P value	0.001			
Sugary-dairy	High	0.16	-0.23	0.56
	Medium	0.15	-0.40	0.70
	Low	0.071	-0.02	0.16
P value	0.372			
Alcohol-trend	High	0.57	0.12	1.01
	Medium	0.59	-0.03	1.22
	Low	0.321	0.21	0.42
P value	0.705			
Veg-trend	High	0.01	-0.38	0.41
	Medium	0.12	-0.43	0.67
	Low	0.18	0.09	0.27
P value	0.267			
Pattern 6	High	0.08	-0.32	0.49
	Medium	-0.31	-0.87	0.26
	Low	0.12	0.03	0.22
P value	0.085			
Pattern 7	High	0.08	-0.35	0.39
	Medium	0.32	-0.05	0.70
	Low	0.09	-0.01	0.19
P value	0.345			

[1] Presented values are adjusted for longstanding illness.

Table 4.18 b- Dietary scores by malnutrition risk among women one-way ANOVA[1] (NDNS).

Dietary patterns		Mean	95% C I	
Healthy	High	-0.17	-0.45	0.11
	Medium	0.04	-0.21	0.29
	Low	0.12ı	0.04ı	0.20ı
P value	0.1			
Traditional	High	-0.44	-0.65	-0.24
	Medium	-0.31	-0.57	-0.05
	Low	-0.25	-0.31	-0.18
P value	0.23			
Sugary-dairy	High	-0.10	-0.35	0.14
	Medium	-0.24	-0.49	0.01
	Low	-0.24	-0.31	-0.17
P value	0.54			
Alcohol-trend	High	-0.36	-0.53	-0.19
	Medium	-0.14	-0.44	0.17
	Low	-0.30	-0.36	-0.24
P value	0.23			
Veg-trend	High	-0.03	-0.28	0.21
	Medium	-0.42	-0.61	-0.24
	Low	-0.06	-0.14	0.03
P value	0.03			
Pattern 6	High	-0.09	-0.30	0.13
	Medium	0.01	-0.22	0.25
	Low	-0.06ı	-0.13	0.02ı
P value	0.84			
Pattern 7	High	-0.19	-0.42	0.04
	Medium	-0.08	-0.36	0.20
	Low	-0.12	-0.19	-0.05ı
P value	0.80			

[1] Presented values are adjusted for longstanding illness.

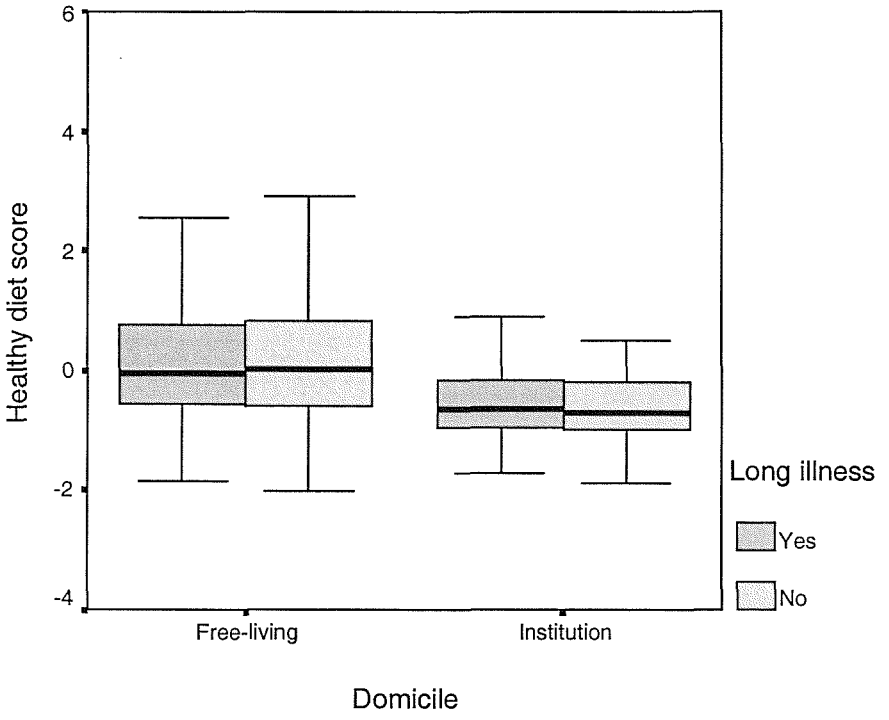


Figure 4. 16- Distribution of healthy diet score by domicile and longstanding illness (NDNS).

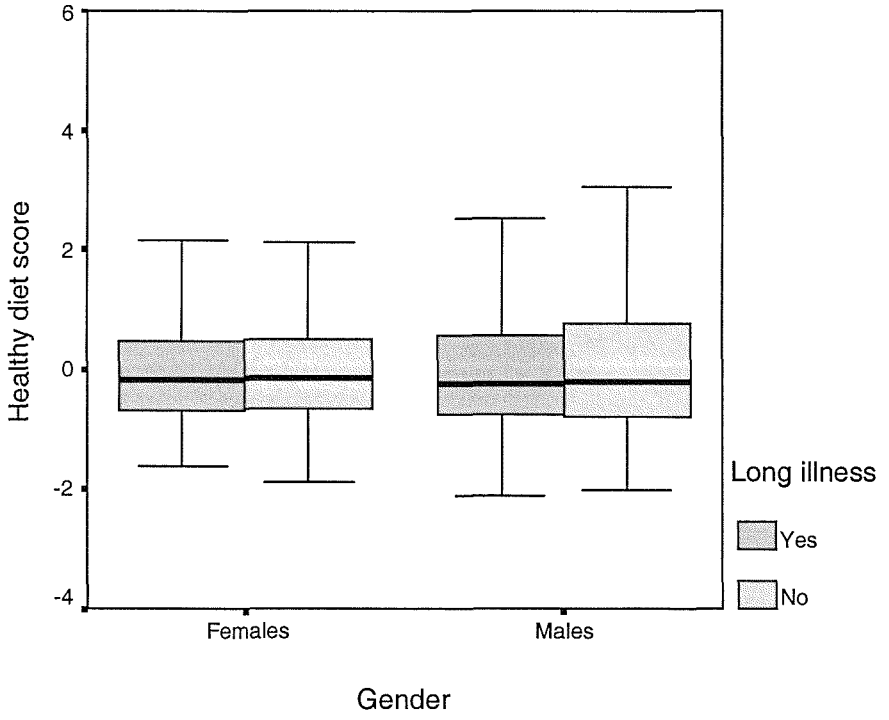


Figure 4. 17-Distribution of healthy diet scores by sex and longstanding illness (NDNS).

Summing up, it appears that except long illness, other variables including; age, smoking, domicile and physical activity are associated with food preference and eating behaviour in the elderly, with rather similar patterns in men and women. These variables are also associated with each other as for example older individuals are less active and less active people are les likely to eat healthy foods.

Figure 4.18 and 4.19 depict the variation of "healthy diet" score by different lifestyle and background variables among institution and free-living samples. Since "healthy diet" followed similar variation patterns in men and women, pool data for sexes is used in producing these figures. Less variability by different background and lifestyle variables is evident in subjects living in institutions compared to those of free-living people.

In order to consider the combined effects of above-mentioned variables on food patterns, a model of analysis of variance (ANOVA) was developed for each dietary pattern, independently. In this method differences in variability of dietary scores between and within subgroups are compared and would be tested for statistical significance. Considering all variables simultaneously in the model would explore the effect of each variable on variation of dietary scores after allowing for the effects of other variables being accounted for. Two-tailed *P* value of F ratio (a ratio of "between groups variation" [mean sum of squares] to "within group variation") would indicate the statistical significance of that variable in explaining the variation of concerned dietary pattern. Using a full factorial model of ANOVA, the main effect of each variable and the interactions between included variables on the dependent variable would be tested for significance.

Analysis was carried out for institution and free-living samples as well as men and women, separately. Scores on each dietary pattern were considered as dependent variable and the categorical variables for age (three age groups; 65-74 y, 75-84 y and >85y), smoking habit (as smoker, never smoker or ex-smoker), health status (whether suffering from a longstanding illness or not), risk of malnutrition (as low, medium or high) and physical activity (very active, moderately active and low active) were entered to the model as independent variables.

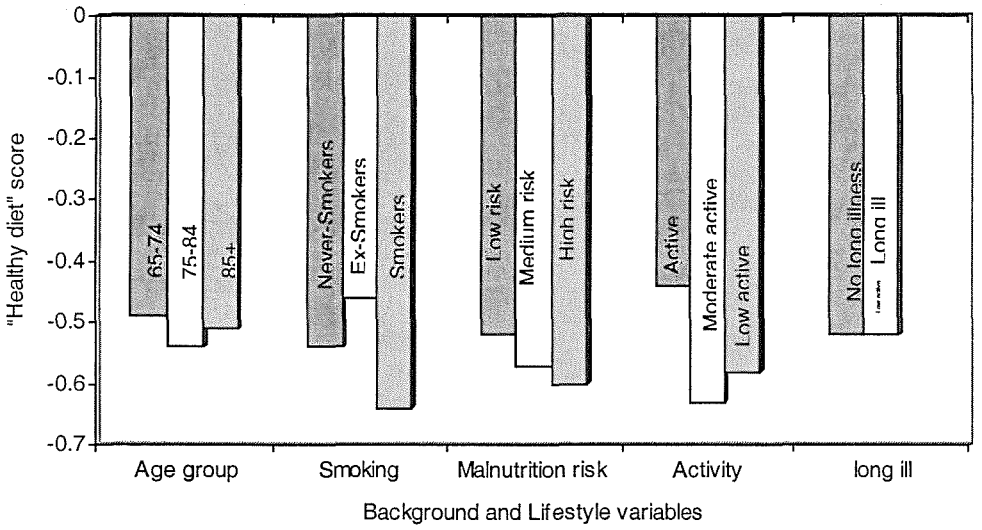


Figure 4. 18-Distribution of healthy diet score by background and lifestyle variables among Institution sample, NDNS.

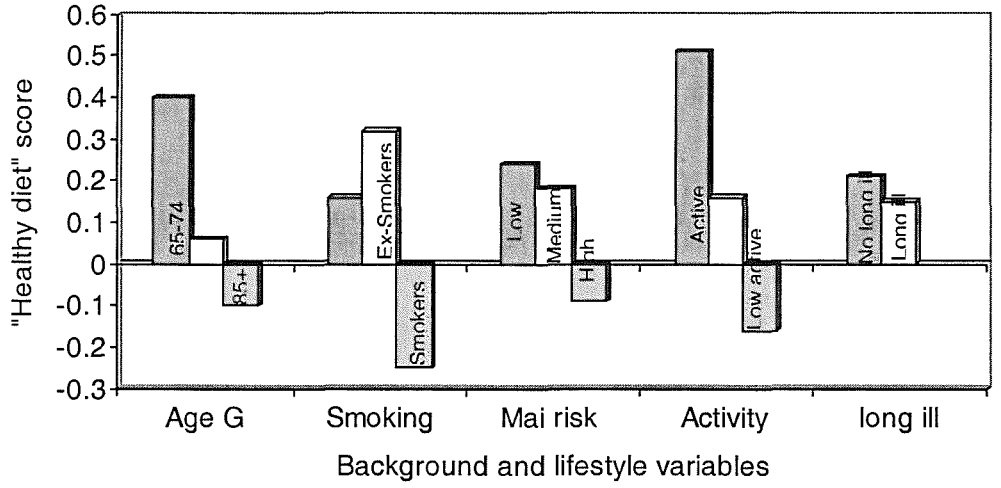


Figure 4. 19- Distribution of healthy diet score by background and lifestyle variables among Free-living sample, NDNS.

Selection of these predictor variables was based on published literature on bone health, as well as their relations to variability of food intakes in our sample, discussed in previous sections. Most of these variables are likely to influence bone health in older population and their effects are partly through effects on eating patterns. Dependent variables were normally distributed across sample subgroups. Since the amount of eaten food may affect the dietary scores, all analyses were controlled for energy intake (MJ).

The results are presented in Tables 4.18-21 for men and women, separately. Presented means of the dietary scores on each pattern, and for subgroups of each variable are adjusted for energy intakes as covariate and other variables in the model, including age, smoking habit, health status and physical activity. Confidence intervals of the means and F ratios are also presented as well as their significance levels, to show the trend for the corresponding dietary score across subgroups. Those patterns, which were significantly affected by selected variables, are discussed in the following sections and are also presented in tables.

4.5.1. The "Healthy diet"

Healthy dietary pattern was related to age, smoking habit, physical activity, and domicile in both sexes (Table 4.19). Risk of malnutrition and long illness were not significantly related to the "healthy diet score". Pairwise comparisons showed that the youngest subjects were significantly different from the two other age groups. Adjusted mean of scores on healthy dietary pattern in the youngest group (65-74 years) was significantly higher than the two other groups (75-84, and over 85). The last two groups however, were not significantly different.

Smoking was also a significant predictor of healthy dietary pattern (Table 4.19). Smokers were less likely to practice healthy diet than nonsmokers.

A positive trend across the activity groups is evident from Table 4.19 in both sexes. Less active subjects were less likely to eat healthy foods. In contrast, more active subjects were more interested to follow this dietary pattern.

The "healthy diet" was more likely to be practiced by free-living subjects in comparison with those living in institutions.

Conducting independent analyses for men and women from free-living and institution samples, showed rather similar results for free-living men and women. For those living in institutions, however, while physical activity was still a significant predictor of healthy diet in men ($F_{(2, 103)} = 4.9$, $P = 0.01$), none of entered variables could predict this dietary pattern, in women.

4.5.2. The "Traditional meat-trend diet"

The second dietary pattern, which was characterized by high intake of bread, coffee and tea and red meat, was more likely to be followed by smoker males, living in private houses ($F_{(2, 540)} = 5.2$, $P = 0.01$). In women none of variables was significantly related to the second dietary pattern. Similarly, in subjects living in institutions this dietary pattern was not explained by lifestyle factors.

4.5.3. The "Sugary food and dairy diet"

This dietary pattern, characterized by high intake of canned fruits, sugary foods and dairy products, was mostly preferred by older age group (over 85 years) in both sexes (Table 4.20). Also, smokers were less interested on this dietary pattern than nonsmokers (ex-and never smokers).

People living in institutions were much more interested on this dietary pattern than free-living ones (Table 4.20). Independent analyses for domicile groups revealed similar results for free-living subjects. For women from institution sample none of variables reached to a significant level but in men living in institutions, malnutrition risk was determinative for practicing this dietary pattern ($F_{(2, 103)} = 4.0$, $P = 0.02$). Because this is a cross-sectional analysis, it is impossible to say males who were practiced more from this dietary pattern were at higher risk of malnutrition or those who were malnourished were more interested on this dietary pattern. However, this pattern was strongly related to energy intake and malnourished persons may need more energy, and, therefore, consumed higher amounts of sugary foods.

4.5.4. The "Alcohol-trend diet"

The fourth dietary pattern was characterised by high intakes of alcoholic drinks, eggs and meat products (Table 4.5). It was followed by smoker men and women (Table 4.21).

Table 4. 19- Men and women- Analysis of variance for scores of the "Healthy diet"; background and lifestyle factors. Covariate: total energy intake (r = 0.23 and 0.45, respectively, *P* = 0.00).

	Men (Grand mean = -0.27)				**Women** (Grand mean =-0.21)			
		Adjusted mean [1]				Adjusted mean		
	F ratio, P value	Mean	95%CI					
Factor: Groups					(*n*)			
Age groups (*n*)	5.6 (2,653) P= 0.00				8.3 (2,618) P= 0.00			
65-74 (266)		-0.08	-0.29	0.13	(230)	0.00	-0.18	0.18
75-84 (280)		-0.36	-0.55	-0.17	(214)	-0.31	-0.49	-0.14
85+ (119)		-0.37	-0.61	-0.14	(186)	-0.33	-0.52	-0.15
Smoking (n)	15.8 (2,653) P = 0.00				8.3 (2,618) P= 0.00			
Never smoker (156)		-0.10	-0.31	0.12	(367)	-0.20	-0.35	-0.05
Ex-smoker (391)		-0.06	-0.24	0.13	(183)	0.01	-0.17	0.19
Current smoker (118)		-0.66	-0.89	-0.43	(80)	-0.46	-0.69	-0.23
Physical activity (*n*)	7.4 (2,653) P = 0.00				9.6 (2,618) P= 0.00			
Low active (210)		-0.48	-0.69	-0.27	(201)	-0.42	-0.60	-0.24
Moderate active (219)		-0.25	-0.46	-0.05	(206)	-0.20	-0.38	-0.03
Very active (236)		-0.08	-0.28	0.12	(223)	-0.02	-0.20	0.15
Domicile (*n*)	50.1 (1,653) P = 0.00				35.7 (1,618) P= 0.00			
Free-living (551)		0.12	-0.06	0.30	(524)	0.09	-0.05	0.23
Institution (114)		-0.66	-0.88	-0.44	(106)	-0.52	-0.73	-0.31

1. Adjusted for energy intake and other variables in the model.

Table 4. 20- Men and women- Analysis of variance for scores of the "sugary-dairy diet"; background and lifestyle factors. Covariate: total energy intake (r = 0.47 and 0.58, respectively, $P = 0.00$).

	Men (grand mean = 0.44)				**Women** (Grand mean 0.07)			
		Adjusted mean [1]				Adjusted mean		
	F ratio, P value	Mean	95%CI					
Factor: Groups				(*n*)				
Age groups (*n*)	5.8 (2,653) P= 0.00				3.2 (2,618) P= 0.04			
65-74 (266)		0.28	0.11	0.46	(230)	-0.01	-0.14	0.12
75-84 (280)		0.43	0.27	0.59	(214)	0.07	-0.05	0.19
85+ (119)		0.62	0.43	0.82	(186)	0.16	0.04	0.29
Smoking (*n*)	7.3 (2,653) P = 0.00				7.1 (2,618) P= 0.00			
Never smoker (156)		0.62	0.44	0.80	(367)	0.16	0.06	0.27
Ex-smoker (391)		0.49	0.34	0.64	(183)	0.18	0.05	0.30
Current smoker (118)		0.22	0.03	0.42	(80)	-0.11	-0.27	0.05
Physical activity (*n*)	1.1 (2,653) P = 0.35				0.1 (2,618) P= 0.88			
Low active (210)		0.47	0.30	0.65	(201)	0.08	-0.05	0.20
Moderate active (219)		0.48	0.31	0.66	(206)	0.06	-0.06	0.18
Very active (236)		0.38	0.21	0.55	(223)	0.09	-0.03	0.21
Domicile (*n*)	58.8(1,653) P = 0.00				143.0(1,618) P= 0.00			
Free-living (551)		-0.01	-0.15	0.14	(524)	-0.35	-0.45	-0.25
Institution (114)		0.90	0.71	1.10	(106)	0.50	0.35	0.65

1.Adjusted for energy intake and other variables in the model.

Table 4. 21- Men and women- Analysis of variance for scores of the "Alcohol-trend diet"; background and lifestyle factors. Covariate: total energy intake (r = 0.27 and 0.21, respectively, *P* = 0.00).

	Men (Grand mean = 0.42)				**Women** (Grand mean =-0.11)			
		Adjusted mean [1]						
	F ratio, P value	Mean	95%CI					
Factor: Groups				(*n*)				
Age groups (*n*)	3.9 (2,653)				0.5 (2,618)			
	P= 0.02				P= 0.95			
65-74 (266)		0.62	0.38	0.86	(230)	0.10	0.25	0.05
75-84 (280)		0.39	0.17	0.61	(214)	-0.11	-0.26	0.03
85+ (119)		0.27	0.01	0.53	(186)	-0.12	-0.27	0.02
Smoking (*n*)	5.4 (2,653)				8.8 (2,618)			
	P = 0.00				P= 0.00			
Never smoker (156)		0.22	-0.03	0.47	(367)	-0.26	-0.38	-0.14
Ex-smoker (391)		0.37	0.16	0.58	(183)	-0.19	-0.34	-0.05
Current smoker (118)		0.69	0.42	0.95	(80)	0.12	-0.07	0.30
Physical activity (*n*)	0.4 (2,653)				0.5 (2,618)			
	P = 0.69				P= 0.63			
Low active (210)		0.37	0.13	0.60	(201)			
Moderate active (219)		0.46	0.22	0.69	(206)			
Very active (236)		0.45	0.22	0.68	(223)			
Domicile (*n*)	1.6 (1,653)				35.7 (1,618)			
	P = 0.20				P= 0.00			
Free-living (551)		0.51	0.30	0.71	(524)	-0.17	-0.28	-0.06
Institution (114)		0.35	0.09	0.60	(106)	-0.06	-0.23	0.11
Long illness (*n*)	0.58 (1,653)				4.4 (1,618)			
	P = 0.48				P = 0.04			
Yes (473)		0.39	0.19	0.58		-0.18	-0.29	-0.07
No (192)		0.46	0.22	0.70		-0.05	-0.20	0.10

1.Adjusted for energy intake and other variables in the model.

Non-smokers (never and past smokers) were less likely to have high scores on this dietary pattern. Among men, age was the other variable explained variation of this eating pattern with no significant difference between free-living subjects and those living in institutions.

Among women, the effect of age and physical activity was not significant, while long illness was significant in predicting of scores on this dietary pattern. Women with long-standing illness were less likely to practice this dietary pattern than others with no long illness. These results were repeated by free-living men and women but among institutionalised subjects none of variables were significant.

4.5.5. The "Vegetarian-trend diet"

This eating pattern was strongly correlated with the consumption of leafy green vegetables, carrots and potato, (Table 4.6). Among men, age and smoking habit were significantly related to this eating habit. Men of the age of between 75-84 and non-smoker males were more interested on this dietary pattern. Among women physical activity was significantly related to the scores of the fifth dietary pattern (Table 4.22). More active females were more likely to practice this dietary pattern. Analyses for free-living men and women revealed rather similar results. However, in free-living women additional to physical activity, risk of malnutrition was also significantly related to the scores on this dietary pattern ($F_{(2,427)}$ =3.0, P = 0.05). Women with medium risk of malnutrition were more interested to follow this eating pattern. In those from institution, none of variables could significantly predict this dietary pattern.

4.5.6. The sixth dietary pattern

The sixth dietary pattern was found in young men and women ($F_{(2,567)}$ =3.7, and $F_{(2,527)}$ =3.5, respectively, P = 0.03). None of other variables were related to this dietary pattern in both sexes. Similar relations was found in free-living sample, while in institution sample none of variable was capable to predict this dietary pattern.

4.5.7. The seventh dietary pattern (margarine and soup)

The seventh dietary pattern characterised by high intakes of soups, margarine and breakfast cereals and low intake of wine, was seen mostly in persons living in institutions in both sexes ($F_{(1,807)}$ = 8.9, P = 0.003 for men and $F_{(1,715)}$ = 3.9, P = 0.05 for women).

Table 4. 22- Men and women- analysis of variance for dietary scores of the "Vegetarian-trend diet"; background and lifestyle factors. Co-variate: total energy intake (r = 0.03, *P* = 0.4 and 0.04,*P* = 0.2, respectively).

	Men (Grand mean = -0.01)				**Women** (Grand mean =-0.15)			
		Adjusted mean [1]						
	F ratio, P value	Mean	95%CI					
Factor: Groups					(*n*)			
Age groups (*n*)	4.7 (2,653) P= 0.01					1.2 (2,618) P= 0.3			
65-74(266)		-0.01	-0.23	0.21	(230)		-0.07	-0.27	0.14
75-84(280)		0.17	-0.04	0.37	(214)		-0.20	-0.39	-0.01
85+(119)		-0.17	-0.42	0.07	(186)		-0.19	-0.39	0.01
Smoking (*n*)	5.6 (2,653) P = 0.00					0.5 (2,618) P= 0.58			
Never smoker (156)		0.20	-0.03	0.42	(367)		-0.09	-0.26	0.07
Ex-smoker(391)		0.03	-0.17	0.22	(183)		-0.15	-0.35	0.05
Current smoker(118)		-0.24	-0.49	0.00	(80)		-0.21	-0.47	0.04
Physical activity (*n*)	0.2 (2,653) P = 0.81					3.5 (2,618) P= 0.03			
Low active (210)		-0.04	-0.25	0.18	(201)		-0.27	-0.47	-0.07
Moderate active (219)		0.03	-0.19	0.24	(206)		-0.17	-0.37	0.02
Very active (236)		0-.01	-0.23	0.20	(223)		-0.01	-0.21	0.18
Domicile (*n*)	0.4 (1,653) P = 0.53					0.0 (1,618) P= 0.95			
Free-living (551)		0.03	-0.16	0.22	(524)		-0.15	-0.30	0.00
Institution (114)		-0.04	-0.28	0.19	(106)		-0.16	-0.39	0.07

1.Adjusted for energy intake and other variables in the model.

Both men and women living in institutions were more interested in this diet, compared to their free-living counterparts. Separate analyses for free-living and institutionalised subjects showed no relation between variables in the model and seventh dietary pattern in free-living subjects from both sexes. Among institutionalized women, age was related to the seventh dietary pattern ($F_{(2,132)} = 3.7$, $P = 0.03$). Averagely, youngest institutionalised women had significantly higher scores on the seventh dietary pattern. The model did not reach to a statistically significant level in men from institutions.

4.6. Prevalence of different eating habits

To calculate the prevalence of seven dietary patterns in the elderly in the UK, each subject was assigned to one of the seven dietary patterns, based on his or her highest dietary score. Using this method, each individual was classified into a specific eating pattern, which was most closely followed by him or her. Since for each subject it was possible to have high scores on each of the dietary patterns, those who were close to two or more eating patterns maybe miss-classified by this method. However, paired t test for the difference between the highest dietary score and the next highest was highly significant, ($t_{(1686)} = 40.8$, $P = 0.00$) suggesting that the issue of "shared individuals" was not significant.

Table 4.23 presents the distribution of dietary patterns by sex and domicile. In total, women were more interested on healthy dietary pattern than men and free-living people were more interested than institution people. The third dietary pattern or "sugary food and dairy diet" was more commonly followed by institution people compared to free-living ones (Figure 4.20). Among the entire sample only 14% practiced healthy dietary pattern and 17% were interested on the "sugary food and dairy diet".

Table 4. 23- Distribution of dietary patterns by sex and domicile. Categorising scheme is based based on the highest dietary score indicating the pattern, which was most frequently practiced by each subject.

	Men					
	Free-living		Institution		Total	
	N	Valid Percent[1]	N	Valid Percent	N	Valid Percent
Healthy	87	13.8%	3	1.5%	90	10.8%
Traditional	129	20.4%	21	10.3%	150	17.9%
Sugary-dairy	48	7.6%	92	45.1%	140	16.7%
Alcohol-trend	104	16.5%	13	6.4%	117	14.0%
Veg-trend	95	15.0%	18	8.8%	113	13.5%
Pattern 6	96	15.2%	31	15.2%	127	15.2%
Pattern 7	73	11.6%	26	12.7%	99	11.8%
Total	632	100%	204	100.0%	836	100%
	Women					
	Free-living		Institution		Total	
	N	Valid Percent	N	Valid Percent	N	Valid Percent
Healthy	147	22.9%	5	2.4%	152	17.9%
Traditional	72	11.2%	9	4.3%	81	9.5%
Sugary-dairy	37	5.8%	104	50.0%	141	16.6%
Alcohol-trend	45	7.0%	4	1.9%	49	5.8%
Veg-trend	113	17.6%	15	7.2%	128	15.0%
Pattern 6	127	19.8%	37	17.8%	164	19.3%
Pattern 7	102	15.9%	34	16.3%	136	16.0%
Total	643	100%	208	100%	851	100%

[1]. Valid percent: the percentage of cases when non-missing values are considered.

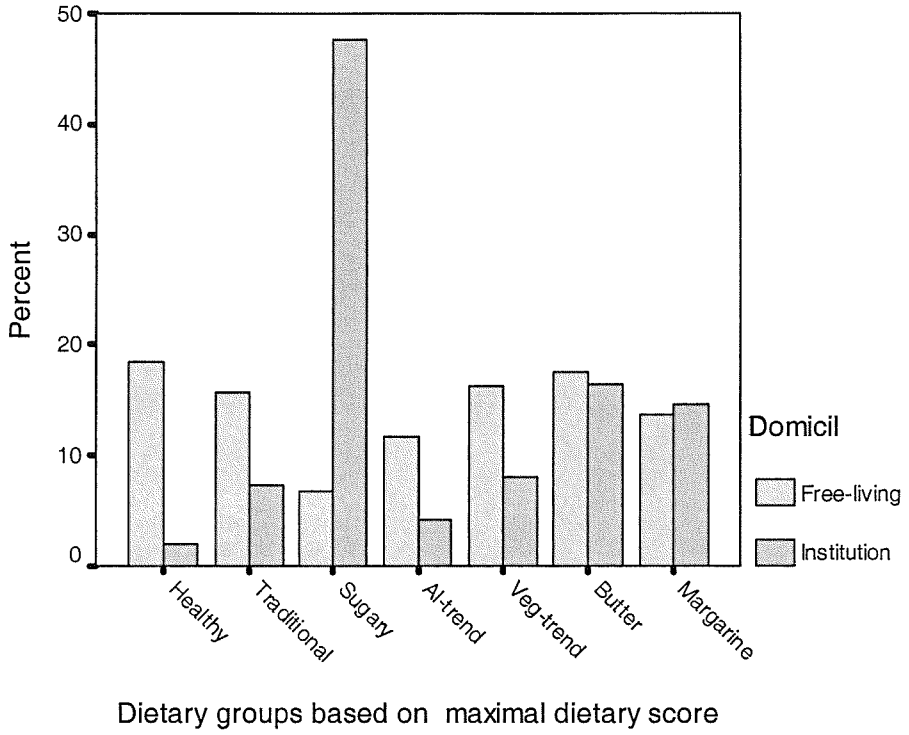


Figure 4. 20- Distribution of dietary patterns by domicile in the elderly in the UK (NDNS)

4.7. Summary

Data of National Diet and Nutritional Survey (NDNS) was used to characterise eating behaviour of the elderly population in the UK. Using principal component analysis, seven different dietary patterns were generated and characterised by disproportionate consumption of different food groups. Identified dietary patterns explained 27.4% of the total variance in amounts of different food groups eaten. For each person factor-loading scores were derived for different dietary patterns and then, the relationships between these dietary scores with nutrient intake and other characteristics of the sample were investigated.

The first dietary pattern, characterised by a high intake of vegetables, fruits, cereals, fish and other seafood, considered as "Healthy diet" and was found to be strongly associated with the intakes of vitamin C, magnesium, potassium and dietary fibre. It was followed significantly by those in younger elderly group (65-74 years), nonsmokers and those who were moderately or very active and living free.

The second dietary pattern characterised by high intakes of breads, coffee, tea, red meat, potato and sausages, and, therefore, was named as "traditional meat-trend diet" or simply "traditional diet". It showed the strongest correlation with protein, energy, zinc, copper and vitamin D intakes among all patterns. A wide range of minerals such as calcium, Mg, phosphorus and potassium was also provided by this dietary pattern. This diet was found mostly in men aged less than 85 years and living free. Smoker males were also interested on this dietary pattern more than others. None of characteristics in women were significantly related to this dietary pattern.

The third dietary pattern was strongly related to the consumption of puddings, canned fruits and dairy products as well as sugar, cakes and pastries and, therefore, was named "sugary-dairy food diet". The third dietary pattern was, like the second, associated strongly with total energy intake (Table 4.9). It was also related to protein, calcium, potassium and phosphorus. It was mostly preferred by older age group (over 85 years) and non-smokers (never and ex-smokers) among men and women. People, living in institutions were more interested on this dietary pattern than free-living people.

The fourth dietary pattern characterised by high intakes of alcoholic beverages, eggs and meat products, and low intakes of dairy products and called "Alcoholic-trend diet". It was highly correlated with alcohol intake and was followed mostly by smokers among men and women with no difference for age and physical activity.

The fifth dietary pattern characterised by high intakes of green vegetables and low intakes of animal products and thus, may be considered as a "vegetarian-trend diet". It was associated with high intakes of dietary fibre, vitamin A, C and potassium but low intakes of protein, calcium and phosphorus. In women activity and in men smoking were related to this dietary pattern. More active females and nonsmoker males were more likely to practice this eating pattern.

The sixth dietary pattern was related to the intakes of low fat spreads and dietary vitamin E. It was preferred by men in lower age group (65-74 years). No relation between this diet and other characteristics was seen in women.

The seventh dietary pattern was related to high intakes of soups, margarine and breakfast cereals and low intake of wine. This pattern was practiced predominantly in institutions. Correlation coefficients between this dietary pattern and energy, protein and some nutrients considered in this study were all less than 0.25 or were not statistically significant. Among women it was commonly seen in youngest age group (65-74 years).

In summary, this chapter was aimed to characterise the eating behaviours or dietary patterns in the elderly in the UK, in order to determine how foods and drinks may be eaten and how may come to diet in the elderly. Advantages and disadvantages of the method of principal component analysis as well as possible limitations of my study of dietary patterns will be fully discussed later in chapter of overall conclusion. However, the dietary patterns identified in the present study were associated with other demographic characteristics and lifestyle variables that may affect bone health, and these associations are consistent with those found in other studies (411). Such associations show that dietary patterns are imbedded in larger health behaviour patterns. This suggests target populations for nutritional education or intervention and points to the importance of considering dietary behaviour in a wider context.

These dietary patterns were used in further analyses to determine the relations between diet and bone health. The methods and the results will be presented in the next chapter.

Some variables such as smoking, physical activity and undernutrition, which are discussed in this chapter, are thought to be related to bone health, as has been discussed in the second chapter. The possible effects of these factors on the relations between diet and bone health are important and will be discussed in the next chapter.

5. Eating patterns and ALP in the elderly in the UK

From published literature, discussed in the second chapter, we concluded that might be a link between overall dietary pattern and bone health.

In chapter four, dietary patterns among elderly population in the UK have been characterized and based on the seven different identified dietary patterns, each subjects was attributed a score on each dietary pattern.

The purpose of this chapter is to determine the relationships between defined dietary patterns and alkaline phosphatase as a marker of bone health with controlling for other bone-affecting variables. The second objective of the study (for primary hypothesis), which was to explore the relationship between dietary patterns and plasma ALP as a marker of bone health, will be addressed in this chapter.

5.1. Alkaline phosphatase as the outcome measure

Alkaline phosphatase (ALP) is referred as a marker of bone metabolism and it was the only marker of bone health, which was available in the data used in these analyses. Higher plasma level of alkaline phosphatase has been considered as a marker of poorer bone health (see chapter two).

Measurement of plasma alkaline phosphatase was possible for 1143 respondents in NDNS; from them 1107 subjects provided a 4-day dietary record. The mean total plasma alkaline phosphatase activity for dietary sample was (94.0 IU/L, SD =62.6) and for the specific assay method used in this survey (see section 3.2.2), 35-110 IU/L is considered to be normal. 34% of subjects (580 persons) had no record for alkaline phosphatase values. To consider the possibility of bias, subjects with missing values on alkaline phosphatase were compared to other respondents. No substantial differences for sex, age, domicile, health status and long-ill period observed. Plasma alkaline phosphatase activity values were log transformed to achieve normality and this transformed value was used in all analyses unless otherwise is stated.

5.2. Dietary variables and ALP

Different statistical methods were used to demonstrate the relationships between alkaline phosphatase, identified dietary patterns, nutrient intakes and other variables, which may affect bone health. In the first stage, correlation coefficients between dietary scores and nutrient intakes with alkaline phosphatase were calculated to explore the extent of the effect of whole dietary package on bone metabolism in comparison with nutrients, which are mostly associated with bone metabolism. Pearson correlation coefficients were calculated for the entire sample and sexes, separately to determine the effect of sex in this regard. Bivariate correlations used to examine associations between each variable with ALP, in separation, and to differentiate confounders from effect-modifiers. Associations were considered as being significant at the 95% ($P<0.05$), 99% ($P<0.01$) or 99.9% ($P<0.001$) confidence levels. Where presented results are shown or described as being "non-significant", indicates that $P>0.05$. Considering these associations, a model of multiple regression analysis is developed to examine the associations between dietary patterns and ALP, while other bone-affecting variables were controlled for. Adjustment for all confounding variables (including dietary patterns, nutrients and lifestyle and background variables) is done by using a full-factorial model, in which all correlations and interactions between different factors with both exposure (dietary scores) and outcome (ALP) as well as between variables themselves will be taken to account. Stratified analysis is used to consider variables which were found to be effect modifier (such as sex).

Because of positive skewness of the distribution of vitamins and dietary copper, these values were logarithmically transformed to achieve normality. Pearson's correlation coefficients between plasma alkaline phosphatase activity, dietary scores and nutrients, which are usually considered to be related to bone metabolism, are presented in Table 5.1. Among men, ALP was highly negatively related to the intakes of potassium, protein, vitamin C and magnesium. The strongest coefficient was that of healthy diet ($r = -0.24$, $P = 0.00$). Alcoholic-trend diet (pattern 4) and the seventh dietary pattern were also related to ALP but with smaller coefficients. Among women none of nutrients, except vitamin C, were correlated with the plasma ALP. Meanwhile, healthy dietary pattern was significantly associated with ALP ($r = -0.1$, $P = 0.03$).

Among the entire sample, ALP was negatively associated with "Healthy diet" (r = -0.17,*P*<0.001) and with the "alcoholic-trend diet" (r = -0.11, *P*<0.001) (positive association with bone health), in fact, the strongest association between dietary variables and ALP was related to healthy diet either in both sexes or in the entire sample.

Table 5. 1 - Pearson's correlation coefficients between dietary variables and plasma alkaline phosphatase activity among men and women[1] (NDNS).

Dietary variables	**Alkaline phosphatase**					
	Men (n = 522)		**Women (n = 447)**		**Total**	
	r	*P* value	R	*P* value	r	*P* value
Vitamin A	-0.07	0.174	0.04	0.441	-0.01	0.653
Vitamin C	-0.18	0.000	-0.12	0.011	-0.15	0.000
Vitamin E	-0.04	0.439	-0.12	0.008	-0.07	0.031
Vitamin D	-0.08	0.087	-0.07	0.125	-0.08	0.012
Zinc	-0.08	0.093	-0.07	0.153	-0.09	0.005
Copper	-0.03	0.549	-0.05	0.316	-0.06	0.050
Magnesium	-0.14	0.004	-0.09	0.048	-0.14	0.000
Calcium	-0.09	0.079	-0.04	0.362	-0.10	0.002
Phosphorus	-0.15	0.002	-0.07	0.125	-0.14	0.000
Potassium	-0.17	0.001	-0.10	0.038	-0.16	0.000
Protein	-0.16	0.001	-0.09	0.048	-0.14	0.000
Total energy	-0.07	0.151	-0.03	0.577	-0.08	0.009
Healthy diet	-0.25	0.000	-0.10	0.028	-0.17	0.000
Traditional diet	0.06	0.267	-0.01	0.893	-0.02	0.545
Sugary-dairy diet	0.00	0.971	0.00	0.951	0.00	0.974
Alcohol-tend diet	-0.17	0.001	0.00	0.975	-0.11	0.001
Veg-trend diet	0.04	0.375	-0.01	0.883	-0.01	0.806
Butter diet	0.00	0.963	-0.05	0.297	-0.03	0.395
Margarine -soup	0.061	0.2021	0.041	0.4001	0.061	0.0501

1 All *P* values are two-tailed and alkaline phosphatase is $\log_e$ transformed.

These data suggest that the relationship between ALP and dietary variables is influenced by sex, greatly. In general, men are more affected by dietary variables compared to women.

Among the whole sample and sexes, the relation between healthy diet and bone health (ALP) was stronger than any nutrient in isolation. It may suggest that the effect of a dietary pattern could not be explained only by its nutrient contents. This would be confirmed when these results be compared with the correlation matrix of dietary patterns and nutrients presented in Table 4.9. Most nutrients, presented in Table 5.1 were related to ALP but the healthy diet was not the only diet related to the intakes of these nutrients. Instead, most nutrients were provided broadly by the first three dietary patterns, from which only

healthy diet is strongly related to ALP. Moreover, "alcoholic-trend diet" (the fourth dietary pattern), which was not strongly related to the intake of any nutrient, was strongly related to ALP with a positive association with bone health (r = -0.12, P =0.00). Note that this dietary pattern was negatively related to the intake of dairy products. On the other hand, the third dietary pattern (sugary-dairy diet), which was strongly related to consumption of dairy products and a vast majority of nutrients, was not related to ALP either in sexes or the entire sample.

5.3. Lifestyle and background variables, Dietary patterns and ALP

Relationships between lifestyle and background variables with dietary patterns, as nutritional exposures, were discussed in previous chapter. This section will examine the effects of these variables on ALP as the outcome measure. Determining the variations of exposures and outcome across subgroups for these characteristics within the sample would help us to develop a model for the relations between dietary patterns and bone health in the elderly.

Relationships between ALP and smoking habit (defined as smokers, ex-smokers and never-smokers), domicile, undernutrition (defined as being at high, medium and low risk of malnutrition according to MAG, discussed in previous chapter) and long illness were examined in men and women, separately. Mean and 95% confidence intervals of ALP are presented in Tables 5.2 and 5.3. Corresponding levels of healthy diet score as the main diet, related to ALP in both sexes and the entire sample, are also presented to compare the variation of outcome and exposure across different subgroups within the sample.

As Table 5.2 shows, the levels of both exposure and outcome varied greatly across the sub-samples for life style and background variables. Smokers had lower intakes of what is said to be healthy foods and had higher levels of plasma ALP (ALP) compared to non-smokers.

One-way ANOVA was used to examine differences in ALP among smoking groups by using transformed values of ALP as dependant variable. Differences between smoking groups were highly significant among men ($F_{(2,577)} = 8.2$, $P = 0.00$). Ex-smokers men had significantly lower levels of ALP indicating better bone health among these subjects in

comparison with the other groups (Table-5.2). Recall that this group had also significantly higher scores on healthy diet. In women however, ALP did not vary among smoking groups (Table 5.3)

Free-living men had lower levels of ALP ($t_{(580)} = 5.14$, $P = 0.00$) and higher levels of healthy diet scores ($t_{(591)} = 11.07$, $P = 0.00$), in comparison with their institutionalised counterparts (Table-5.2). The effect of domicile on dietary scores among women followed a similar pattern, but its associations with ALP were statistically significant (Figures 5.1).

Table 5. 2- Healthy diet scores and alkaline phosphatase level by age and life-style variables among men (n =582).

	Alkaline phosphatase			Healthy diet score[1]		
	Mean	95%CI		Mean	95%CI	
Smoking						
Never smoker	89.2	81.9	96.6	0.40	0.15	0.66
Ex-smokers	81.7	78.1	85.3	0.39	0.23	0.55
Smokers	101.5	88.6	114.4	-0.24	-0.48	0.00
P value	0.000			0.001		
Domicile						
Free-living	84.4	81.4	87.4	0.40	0.28	0.53
Institutionalised	106.2	86.7	125.8	-0.61	-0.81	-0.41
P value	0.000			0.000		
Long illness						
Long ill	86.7	83.3	90.0	0.26	0.13	0.40
Healthy	88.8	78.6	99.0	0.29	0.05	0.52
P value	0.956			0.341		
Physical activity						
Low active	92.4	85.6	99.2	-0.16	-0.36	0.04
Moderate Active	86.1	81.8	90.4	0.25	0.06	0.43
Very active	84.8	77.3	92.4	0.59	0.38	0.80
P value	0.052			0.000		
Age group						
Age 65-74	83.8	80.6	87.0	0.50	0.35	0.66
Age 75-84	90.1	81.2	99.1	0.00	-0.20	0.19
Age 85+	101.0	83.3	118.7	-0.37	-0.69	-0.04
P value	0.031			0.000		
Malnutrition risk						
High	89.0	77.4	100.6	-0.13	-0.65	0.38
Medium	77.6	61.2	93.9	0.13	-0.36	0.62
Low	86.4	82.3	90.5	0.35	0.22	0.49
P value	0.186			0.183		

[1] Adjusted for energy intake

Physical activity was also related to both exposure and outcome in men (Table-5.2). Three activity groups were significantly different in terms of healthy diet scores. More active

men were more likely to eat healthy foods. Rather similar trend but in an opposite direction was seen for ALP across the groups of physical activity. Multiple pair wise comparisons (Tukey test in SPSS) using transformed ALP value showed that low active subjects were significantly different from very active ones in terms of plasma ALP, but the difference between these two groups with the moderate active group was not statistically significant (Table-5.2). Activity index was significantly correlated with ALP (r = -0.17, $P<0.01$) and healthy diet score (r = 0.34, $P = 0.00$) in the whole sample. Results for sexes in separation are presented in Table 5.4. Rather similar patterns of relationships between physical activity and both outcome and exposure was seen in women, but with less pronounced effect, compared to that of men (Table 5.4).

Table 5. 3-"Healthy diet" scores and alkaline phosphatase level by age and life-style variables among women (n =525),NDNS.

	Alkaline phosphatase			**Healthy diet scores**		
	Mean	95%CI		Mean	95%CI	
Smoking						
Never smoker	91.2	85.3	97.0	0.03	-0.07	0.14
Ex-smokers	89.1	85.0	93.2	0.35	0.19	0.51
Smokers	90.3	84.3	96.3	0.11	-0.29	0.08
Domicile						
Free-living	87.7	83.2	92.2	0.33	0.23	0.42
Institutionalised	98.1	92.3	103.9	-0.50	-0.59	-0.41
Long illness						
Long ill	90.6	85.8	95.4	0.08	-0.02	0.17
Healthy	90.1	85.2	95.0	0.18	0.01	0.34
Physical activity						
Low active	99.8	86.3	113.3	-0.27	-0.40	-0.14
Moderate active	86.7	83.3	90.1	0.04	-0.10	0.19
Very active	87.0	83.3	90.8	0.43	0.30	0.57
Age groups						
Age 65-74	84.4	81.7	87.1	0.48	0.36	0.61
Age 75-84	91.6	87.9	95.3	-.14	-0.27	0.02
Age 85+	103.1	85.8	120.3	-0.32	-0.47	-0.17
Malnutrition risk						
High	89.6	81.0	98.3	-0.90	-0.40	0.22
Medium	90.6	80.4	100.9	0.06	-0.28	0.39
Low	88.1	83.7	92.5	0.22	0.12	0.31

Among men, long-standing illness was not related to both outcome and exposure as the levels of healthy diet score and ALP did not vary between ill and non-ill individuals, significantly (Table 5.2). It was not also a significant factor in relations between diet and bone health among women (Table 5.3).

Age was also related to both ALP and healthy diet scores in men (Table 5.2). Men within the youngest age group (65-74) had significantly higher scores on healthy diet and lower ALP in comparison to the oldest age group (85+). Both healthy diet score and ALP were overlapped between the middle age group and both youngest and oldest group. Among men age was positively related to ALP (Rs = 0.10, $P = 0.01$) and healthy diet score (R_s =-0.22, $P<0.001$). Among women, although age as a continuous variable was not significantly related to ALP (Rs = 0.07, $P = 0.11$), differences between age groups were statistically significant ($F_{(2.524)} = 3.09$, $P = 0.05$). The youngest group was significantly different from the oldest one with respect to the plasma ALP(Table 5.3).

Differences between subjects by the risk of malnutrition are also presented in Tables 7.2 and 7.3. As was expected, they were different in terms of dietary intakes (healthy diet scores) ($F_{(2.1307)} = 5.7$, $P = 0.00$) in the entire sample. Those at higher risk of malnutrition had significantly lower healthy diet scores in comparison with those at lower risk, but those within the medium risk group were not significantly different from the other two groups. Similar pattern was found in men, while in women differences of dietary scores were not significant. Their differences in plasma ALP however, were not statistically significant, either in total or sexes in separation.

Summing up, among men, life-style variables including smoking habit, physical activity, domicile index were related to both exposure and outcome and seems to act as confounding factors for the relations between dietary patterns and bone health. Relations of dietary scores with smoking, activity and domicile among women followed a similar pattern of men with less pronounced associations. With respect to outcome (ALP) however, domicile and smoking had no effect among women.

Being ill for a long time did not associate with the level of ALP and healthy diet score and was not related to any of other life-style variables and, therefore, it appears to have no effect on the relationships between diet and bone health in both sexes. Malnutrition risk was related to dietary intake but has no effect on ALP in men and women.

Table 5. 4- Pearson's correlation coefficients between ALP[1] with age and physical activity among men and women.

	Activity index		
	Men	Women	Total
Healthy diet score			
r	0.35	0.32	0.34
P value	0.00	0.00	0.00
ALP			
r	-0.19	-0.14	-0.17
P value	0.00	0.00	0.00

1.Log-transformed value is used

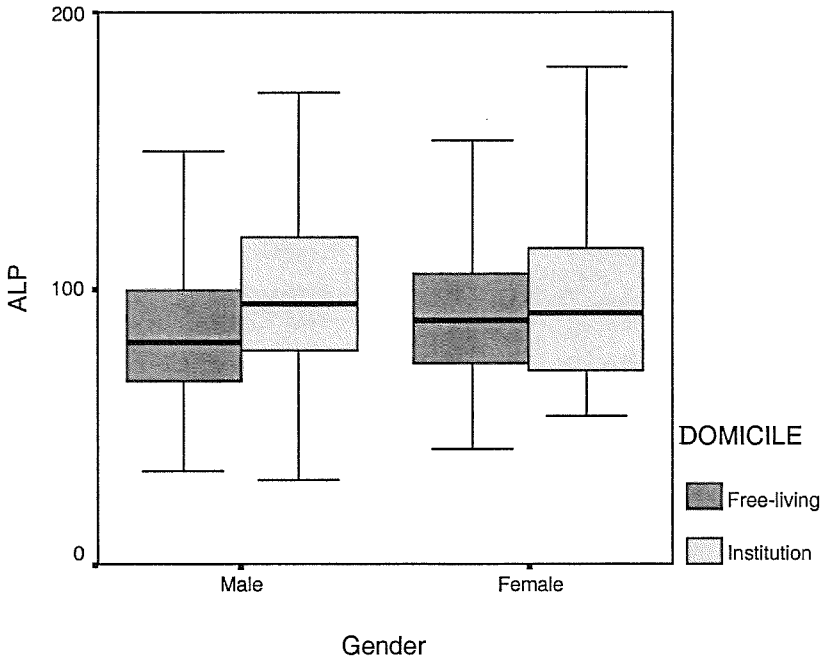


Figure 5. 1- Distribution of ALP among men and women by domicile index.

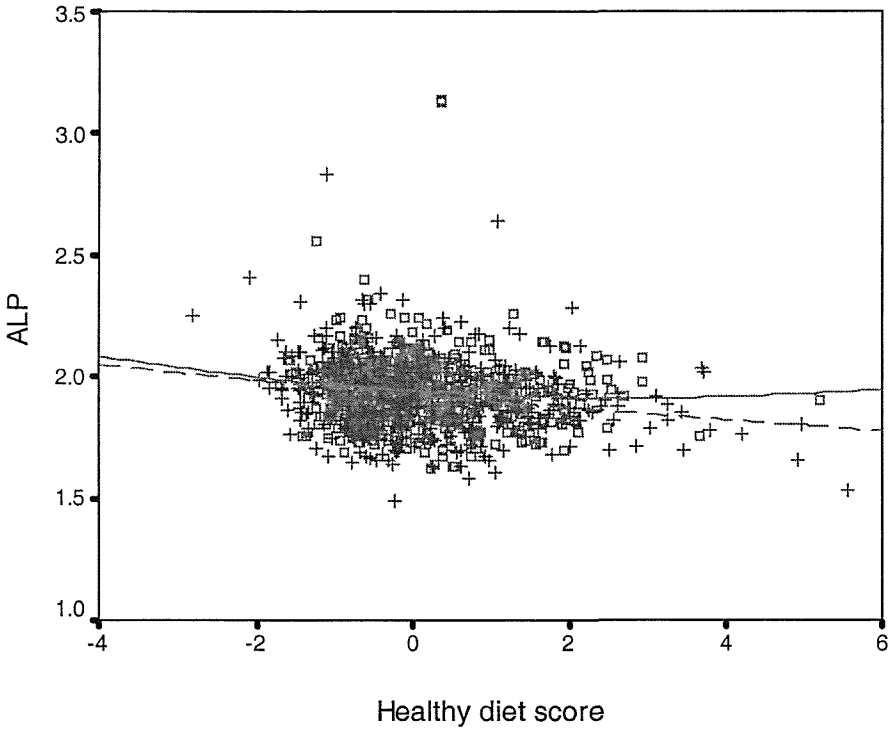


Figure 5. 2- Plots and regression lines between Healthy diet score and alkaline phosphatase among men (+,– – –) and women (□, ——), NDNS.

5.3.1. Multiple regression analysis

Based on the results of the initial analyses, a model of multiple regression analysis was developed to examine the associations between dietary patterns and ALP while other bone affecting variables were controlled for. Factors included in the model were those for which distributions varied both by exposure (dietary intakes, represented by dietary scores) and outcome (serum ALP), or those that are risk factors for bone health, ie. smoking, age, long illness etc, according to the current literature (see Chapter 2). The scatter diagrams showed that the relations between dietary patterns and ALP were linear (Figure 5.2).

Multiple regression analysis was used to model the relationships between dietary patterns and plasma alkaline phosphatase. In this method the combined effect of different variables on prediction of a dependent variable would be explored. The effect of each variable is

also tested for significance when the effects of other variables are controlled for. Partial regression coefficients (unstandardized coefficients or B) estimate the amount of change that occurs in the dependent variable for a one-unit change in the concerned independent variables, while the effects of other variables in the model are controlled for. To compare the relative importance of each independent variable to predict the dependent variable, these coefficients would be standardized by multiplying each by the product of dividing the standard deviation of the relevant independent variable by the standard deviation of the dependent variable. These standardized coefficients are known as Beta weights. They tell us how many standard-deviation units the dependent variable will change for a one standard deviation unit change in the independent variable. R square (R^2) tells us about the percentage of variance in the dependent variable explained by the model. Changing in the value of R^2 due to the inclusion of each independent variable in the model by stepwise approach shows the importance of that variable to explain the variance of the dependent variable.

Our dependent variable in this model was the plasma alkaline phosphatase activity (after being logarithmically transformed to achieve normality) and independent variables were included: dietary scores as nutritional variables and age, smoking, physical activity, domicile, long illness, socio-economic class and the risk of malnutrition as non-nutritional variables. Categorical data were entered the model by creating a set of dummy variables for each (K-1 dummy variables). A dummy variable is a dichotomous variable with the values of 1 (indicating a character) and zero (indicating the lack of that character). For example; for entering the activity groups in the model two dummy variables (K-1) were created; one for low active group (as being low active or not) and the other for very active group (as being very active or not). Dummy variables were created for activity groups, malnutrition risk, smoking habit, domicile, socio-economic class and long-illness. Therefore, all categorical variables entered the model were dichotomous with values of 0 and 1. All interval variables were normally distributed. By Entering the total energy intake in the model the dietary intakes were adjusted for energy intake (426).

The results of multiple regression analysis for men are presented in Table 5.5. The strongest predictor of bone health among men was the healthy dietary pattern, which was nega-

tively related to the plasma alkaline phosphatase activity (beneficial positive association with bone health). The fourth dietary pattern (alcoholic-trend diet) was also related to bone health. Excluding 6 subjects with outlier residual values of ALP (detecting by case wise diagnostic in SPSS) did not change the R^2 and ΔR^2 for healthy diet, substantially. The model including all these variables was accounting for a combined 11 % of the variation in plasma alkaline phosphatase activity ($P<0.05$). Plots of residual distribution confirmed that the assumptions of the analysis (linearity of relations and homogeneity of variance) have been met.

Total energy intakes, other dietary patterns, long-ill index, age and activity index were excluded from the analysis because of non-significant effects. The healthy dietary pattern explained 7.2% of variation in alkaline phosphatase. Other variables explained progressively smaller amounts of the variation in plasma ALP among men.

In summary, these results showed the importance of healthy dietary pattern for bone health among elderly British men. Age and activity did not predict bone health among this population.

Using the same model among women showed different results (Table 5.5). The only predictor of bone health among them was the healthy dietary pattern, which was accounted for 2.5% of variation in alkaline phosphatase ($B = -0.02$, $\beta = -0.14$, $R^2 = 0.023$, $P = 0.00$). Analysis was performed for 525 subjects because data on alkaline phosphatase was missed for others (value of alkaline phosphatase was not recorded for 326 subjects). Excluding 6 women who were using medicines for obstetric and gynecologic treatment did not change the results.

Since subjects with missing values and those with valid values of alkaline phosphatase were approximately similar in other variables, in order to increase the number of subjects in the study, missing values were substituted with the mean. Using these substituted values after logarithmic transformation, the predictive variables remained unchanged among men and women.

Independent analyses for free-living and institution subgroups also found the healthy diet as the strongest predictor of ALP in both sexes in free-living people. However, in free-

living women the sixth dietary pattern was also a significant predictor ($\Delta R^2 = 0.013$, B = -0.02, $\beta = -0.14$, $P = 0.01$). Among institution sample none of entered variable could predict ALP, significantly.

Table 5. 5- Stepwise multiple regression analysis for men (n = 581) and women (n = 525): Partial regression coefficients (B), standardized regression coefficients (β), changes in R^2 (ΔR^2) and statistical significance of predictors of plasma ALP; predictors: smoking, age, energy intake, long-standing illness, domicile, malnutrition risk (defined by MAG tool), physical activity, socioeconomic class and seven dietary patterns (NDNS).

Variables	B	95% CI of B		β	R^2	Adjusted R^2	ΔR^2	df	*P* value
Men									
Healthy diet	-0.02	-0.03	-0.01	-0.19	0.064	0.061	0.064	397	0.000
Alcohol-trend diet	-0.01	-0.02	0.00	-0.16	0.121	0.114	0.024	395	0.002
Women									
Healthy diet	-0.02	-0.03	-0.01	-0.15	0.021	0.019	0.021	500	0.001

Excluding those who were suffering from disability or morbid diseases such as cancer, kidney diseases and endocrine diseases, increased the importance of healthy diet in explaining the variance of ALP in both sexes (improved ΔR^2 by small amounts). But the model was still insignificant in institution sample.

In order to examine the importance of nutrients in predicting plasma ALP in comparison with dietary patterns, nutrients which are said to be related to bone health, ie. zinc, phosphorus, calcium, potassium, protein and log-transformed values of vitamin D, C, E, A and copper, were entered the model simultaneously with the dietary scores. All nutrients dropped from the model because of non significant effect in men and women, suggesting that the dietary patterns are more important in predicting ALP than any nutrient in isolation.

Under-reporting is one of problems in dietary surveys. In order to examine whether my results are affected by under-reporting of dietary intake, the model of multiple regression analyses were repeated for both sexes with excluding those who were more likely to under-report their dietary intakes. Under-reporters were defined as having an energy intake to basal metabolic rate ratio (EI/BMR) of less than 1.2 as described by Goldberg and coworkers (511). Approximate values for basal metabolic rate (BMR) of participants were estimated using standard simple regression based on body weight and the age and sex of

the participant (509;510). 41% of free-living men and 59% of free-living women reported values lower than 1.2. In institution sample 28% of men and 26% of women had calculated values of less than 1.2. Using the same model of multiple regression analysis in men after excluding subjects with EI/BMR <1.2 did not change the obtained results, substantially (R^2 =0.07, adjusted R^2= 0.06, df = 206, P = 0.000). However, in women, the effect of healthy diet did not remain significant when subjects with EI/BMR <1.2 were excluded from the analysis. These results may indicate that the early results of the association between healthy diet and bone health in women were biased by under-reporters or excluding those who were likely to under-report their dietary intakes reduced the power of the study to explore the association between healthy diet and ALP in women. In order to increase the sample size, subjects were selected regardless of the EI/BMR ratio and then EI/BMR entered the model as continues or categorical variable. The early results obtained and EI/BMR ratio dropped out of the model because of non-significant effect. However, levels of energy intakes less than 1.2 times of BMR are unlikely to meet the energy requirement and thus, the low-recorded energy might be due to misreporting or modifying participants' diet during the recording period and therefore do not representing usual dietary habits of the subjects..

5.3.2. Binary logistic regression analysis

In order to assess the effect of "healthy diet", as the main dietary pattern related to ALP, on the risk of having high level of plasma ALP, I constructed a model of logistic regression for men and women, independently. I used sex and domicile-specific median of ALP to dichotomise the distribution of plasma ALP. Median value was selected arbitrarily in order to increase the statistical efficiency of the analysis by decreasing the effects of data distribution (skewness) and having rather equal number of cases (defined by plasma ALP of higher than median) and controls (defined by ALP lower than median). Healthy diet scores were classified into sex and domicile-specific quartiles, which were modeled with indicator variables, and individuals in the lowest quartile category served as the reference category. Categorical data of age, smoking habits, physical activity, malnutrition risk, longstanding illness and domicile were also modelled as indicator. Analyses were controlled for energy intake and scores on six other dietary patterns (patterns 2-7). χ^2 test for

trend was used to examine the statistical significance of the trend of odds ratios across the quartiles of healthy diet scores (427).

Table 5.6 presents the results of the binary logistic regression analyses for ALP across the quartiles of healthy dietary scores. Healthy diet score was inversely associated with the prevalence odds ratios of high plasma ALP (defined by higher or lower than median value of the ALP distribution). From the lowest to the highest quartile of "healthy diet" intake, the prevalence odds ratios of having high levels of ALP decreased by more than half in women and by two third in men with a statistically significant trend in both sexes (*P* for trend <0.001). Separate analyses for men and women in free-living sample produced similar results. In institution sample, however, because of small sample size and wide confidence intervals, analyses were not informative.

Smoking was also associated with the prevalence of odds ratio of high plasma level of ALP among men but in women. Smoker men in comparison with non-smokers had an OR of 2.0 (95% CI, 1.2- 3.1). Other variables in the model were not significantly associated with the prevalence of high ALP. Subjects with highest odds ratio were more likely to have lower intakes of fruits (15g/d v.108g/d), vegetables (16g/d v.66g/d), chicken and turkey (7g/d v. 31g/d), fish and shellfish (21g/d v.40g/d). Other characteristics of the subjects with respect to the quartiles of the healthy diet score are presented in Table 5.7.

Table 5. 6- Prevalence odds ratios * (95% CIs) of high ALP (defined by median of ALP) and the median values of ALP by quartiles of healthy diet score in men and women (NDNS).

	Quartiles of healthy diet scores			
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Men				
N (cases/ controls) §	75/52	55/64	65/65	54/89
OR† (95% CI)	Reference	0.66 (0.3-1.1)	0.85 (0.4-1.4)	0.40 (0.2-0.7)
ALP (IU/L)	98.7	86.3	88.0	84.2
Women				
N (cases/ controls) §	50/52	50/46	56/64	45/79
OR† (95% CI)	Reference	0.89 (0.5-1.5)	0.72 (0.4-1.2)	0.46 (0.3-0.8)
ALP (IU/L)	93.0	91.4	94.8	83.7

* Odds ratios are adjusted for age, energy intake, smoking habit, physical activity, domicile, long-standing illness and malnutrition risk.

† *P* for trend <0.01

§ Cases and controls were defined by having a value of ALP of higher and lower than the median, respectively (86.8 IU/L)

Table 5. 7- Mean intakes of foods of healthy dietary pattern g/d (SD), age and percentage of smokers by quartiles of healthy dietary scores (NDNS).

	Quartiles of healthy diet score			
	First	Second	Third	Forth
Women				
N	196	217	238	200
Salad and other vegetables	15.0(17.3)	23.4 (21.9)	34.7 (30.3)	65.2 (51.1)
Fresh fruits	14.6 (26.4)	33.0(37.2)	55.3(52.1)	108.4(89.5)
Chicken and turkey	6.1(13.2)	11.4(18.3)	17.1(24.7)	29.8(38.8)
Fish and shellfish	19.8 (20.9)	25.7(25.5)	28.8(25.4)	34.0(29.9)
Age (SD)	83 (8)	80 (8)	79 (9)	76 (8)
% of smokers	19%	14%	10%	11%
Men				
N	225	205	184	222
Salad and other vegetables	17.3 (18.4)	27.9 (28.2)	37.1 (34.0)	67.5 (50.7)
Fresh fruits	15.9 (28.3)	27.3 (42.3)	55.7 (59.4)	108.9 (84.8)
Chicken and turkey	8.3 (13.9)	10.8 (19.7)	18.9 (27.5)	33.3 (39.6)
Fish and shellfish	23.3 (27.5)	28.6 (27.5)	33.1 (30.6)	47.0 (40.8)
Age (SD)	79 (7)	78 (8)	77 (8)	75 (7)
% of smokers	48%	35%	22%	19%

5.4. Summary

Relationships between plasma alkaline phosphatase and dietary patterns were investigated by analyses presented in this chapter. Based on the results of these analyses, dietary patterns may be more important in predicting bone health than any nutrient on its own. Presented results suggested that there was an added benefit of the dietary pattern, above and beyond that seen of the nutrients included in this analysis. The pattern analysis, therefore, can be referred to as a valuable tool for nutritional strategies in the context of public health nutrition. From a policy perspective, an approach that describes dietary patterns is most helpful because people do not eat nutrients and therefore any messages are most easily understood expressed as foods, rather than nutrients. This suggests that attempts to improve bone health may be more successful if they emphasis dietary patterns rather than nutrients. Allied to this, it was shown that a diet, which is characterised by high intakes of fruits, vegetables, cereals, fish and other seafood, and low intakes of sugar and puddings and broadly complies with the current guidelines in the UK for a "healthy diet", is associated with lower plasma alkaline phosphatase (ALP) in the elderly. This effect was significant even after controlling for energy intake and a number of bone affecting variables, including; physical activity, smoking, malnutrition risk, domicile, long illness, sex, age

and nutrients such as vitamins A, D,C,E,K, protein, calcium, phosphorus, magnesium, zinc, copper, potassium and fibre. These results provided support for the healthy dietary guidelines in the UK as being beneficial for bone health in the elderly. Forth dietary pattern characterised by high intakes of alcoholic beverages was also related to ALP in a negative manner. Its effect however, was significant only in men. Other dietary patterns permanently stood out of being significantly better or worse in relation to bone health. However, it is not clear from the analyses undertaken in this chapter what the critical components of a healthy diet are for bone health; all that can be said is that by examining the pattern of diet provides additional information that can not be as yet identified by a nutrient analysis.

The results are consistent with other studies that investigated the association between dietary patterns and other health-related conditions such as cardiovascular risk factors and cancer diseases. Hu et al (412) showed that a diet similar to the "healthy diet" in our study (high intake of vegetables, fruit, legumes, whole grains, fish, and poultry) was associated with decreased risk of CHD in old adults. Also, Fung et al (416) reported the protective association between a healthy dietary pattern (a diet with higher intakes of fruit, vegetables, whole grains, and poultry) and plasma biomarkers of CVD and obesity (positive correlation with plasma folate and inverse correlation with insulin and homocysteine). The results also agreed with the studies that reported a protective effect of a diet rich in fruits and vegetables on bone health (evaluated by BMD and bone markers)(216). Dietary patterns derived in this study were qualitatively similar to those of other studies, obtained by similar approach of principal component analysis (410; 412;416; 418; 428429-431). Also, principal component analysis has been shown to have good reproducibility and validity in assessing dietary patterns (430).

It is important to note that the "healthy diet" identified in this study, was broadly compatible with the guidelines of the healthy eating pattern in the UK (448). Consumption of variety of foods, having a plenty of fruits and vegetables in the diet, eating plenty of foods rich in starch and fibre, avoiding the consumption of too much sugary foods and drinks and not eating too much from foods that contain a lot of fat are the key points of the guidelines which are compatible with the characteristics of the "healthy diet" identified in

the present study. The results support these guidelines in the UK and showed their value for bone health in elders.

According to the presented results the forth dietary pattern, characterised by higher intakes of alcoholic drinks, eggs, meat products and nuts and seeds (including fruit and nut mixes) was also associated with lower ALP in men only. However, in men, it explained only 2% of variation of ALP and in women the association was not significant. Published studies on alcohol consumption and bone health have produced inconsistent results (see section 2.2.7). Positive association between bone density and alcohol intake was reported in a 12- year prospective study by Holbrook et al (215) and was shown in Framingham Heart Study Cohort by Felson et al (217). These results were also repeated in a cross-sectional study (216). Contrasting to these results, case-control studies showed negative association between alcohol intake and bone mineral situation (218; 219). Note that these effects are different from those of studies with osteoporotic fractures as outcome. Studies in which fractures were used as outcome, consistently reported negative association between alcohol intake and risk of fracture (159;205; 220-222), an effect that may be due to the neuromuscular consequences of alcohol consumption and increasing fall risk. However, Holbrook (215) concluded that social drinking is associated with higher bone mineral density and COMA accepted a sensible alcohol drinking as a part of healthy dietary guidelines in the UK (448). Positive association between bone mineral and alcohol intake may be because of augmentative effect of alcohol on oestrogen (217). However, as stated earlier, the negative association between alcohol-trend diet with ALP is due to the diet in total. From the analyses we can not say this association is because of alcohol or other components of this diet.

However, as has been discussed in Chapter two, bone mass is influenced by skeletal size which is reflected by body weight and height. Moreover, these two measures of body dimension may represent the effects of nutritional exposures in early life, which are thought to be important in predicting bone health in the elderly. Possible interaction between these dietary patterns and body size is the subject of the next chapter.

6. Eating patterns, body size and ALP

Interaction between dietary patterns and body size is the subject of this chapter. This chapter is aimed to address the objective of the secondary hypothesis, which is investigation on the effects of body size on the relations between diet and bone health. Body size may be considered as its crude measures such as bodyweight and height or a ratio between these two measures, such as BMI.

6.1. Body size, diet and ALP

Weight and height are two measures of body size, which are determinant for bone health but with different underlying mechanisms (40;41). Height is a measure of the skeletal size and skeletal size is determinant for bone mineral status as it provides an envelope, in which bone tissue must be laid-down. Weight on the other hand, is related to the body composition, in terms of fat and lean body mass, which are important for hormone profiles and mechanical strains due to muscle function, respectively (see chapter two). Furthermore, body weight determines the mechanical loading strain on the skeleton, which is important for bone remodelling and bone strength. In some part, the associations between height and weight with bone health may be related to the past exposures reflected by these measures; taller height due to more rapid growth is associated with an earlier puberty and therefore a longer exposure to adult concentrations of sex hormones (270), which are regarded to as beneficial factors for bone mineral status. Furthermore, both measures and in particular height, are regarded as proxies of early nutrition and childhood growth and development, with taller people more likely to have been nourished optimally during their growth period than shorter ones (459), notwithstanding the major influence of heredity (heritability values of 0.75-0.78 (455)). In this respect, because height is related to the nutritional and other environmental exposures during childhood and being tracked from that time, it may represent an important marker of childhood influences on chronic diseases and bone health in adults. Additionally, childhood diet may not only influence the growth in body size but also the concentration of insulin-like growth factor-I (458), which is an important stimulator for osteoblast replication and enhance the collagen synthesis (see chapter 2).

Body mass index (BMI; $W_{kg}/S^2{}_m$), as a ratio of weight and height, is regarded as an index of total body fat in men and women (456). The effect of BMI on bone health may be mostly due to the effect of fat body mass and peripheral conversation of androgen to oestrogen (441) (see chapter 2). BMI is independent of height but is highly associated with weight (440). In adults BMI is not related to body size at birth (40;463)and is weakly related to its values in childhood (268;269), and therefore, in comparison with weight and height, BMI may not be considered as a proper proxy of nutritional and environmental exposures during growth. However, due to differences between individuals in fat distribution and particularly in the elderly that losing muscle mass is a an important determinant of body composition (457), BMI may not properly represent the body composition in the elderly (462).

Regarding to the differences between height, weight and BMI in their associations with bone health, and their biological correlations, different procedures were used to determine their correlations with exposure (diet), outcome (ALP) and other variables that may act as confounder in the study population.

Initial analyses on the NDNS data showed that BMI, weight and height may reflect different variables in respect to bone health, as their relations with both exposure of interest (dietary patterns) and outcome (ALP) were different.

Table 6. 1. Spearman correlation coefficients between BMI, weight and height with seven dietary patterns (NDNS), all P values are 2-tailed.

	BMI	Weight	Height
Healthy diet	0.138	0.222	0.162
P value	0.000	0.000	0.000
Traditional diet	0.053	0.240	0.347
P value	0.109	0.000	0.000
Sugary-dairy diet	-0.141	-0.046	0.082
P value	0.000	0.158	0.011
Alcohol-trend diet	0.015	0.154	0.232
P value	0.654	0.000	0.000
Veg-trend diet	0.016	0.094	0.125
P value	0.630	0.004	0.000
Low fat spread	0.108	0.122	0.084
P value	0.001	0.000	0.010
Margarine	0.061	0.074	0.064
P value	0.063	0.023	0.050

As Table 6.1 shows the correlation coefficients between seven different dietary patterns and BMI, body weight and height were different. For healthy diet, correlation of BMI was weaker than that of weight and height, and for alcohol-trend and vegetarian-trend diets, BMI was not related to these dietary patterns, while both weight and height were strongly correlated. Similar pattern of correlation was found for most other dietary patterns. Given the association between dietary patterns and lifestyle and background variables (see chapter four), this bivariate analysis indicated that these three measures may represent different variables in our population. Further analyses conducted to differentiate underlying variables which may be different regarding to the dietary patterns. These analyses are presented in Appendix 2. As Table 1 in Appendix 2 shows, BMI was not associated with the outcome measure (ALP), activity score, energy intake and household social class, ascribed on the basis of the occupation of the head of household, while weight and height were strongly associated with these variables. Age was negatively associated with BMI, weight and height but the correlation coefficients for weight and height were far stronger than that of BMI. However, one way ANOVA (using activity group as predictor) indicated a significant difference between low-active and very active subjects for BMI ($F_{(2,1320)}= 5.5$, $P = 0.004$), but the difference of BMI across the socio-economic class was not statistically significant ($F_{(3,1063)}= 1.6$, $P = 0.187$).

Further analyses on weight and height revealed that these two variables may reflect different levels of other variables related to bone health. Sex and domicile-specific thirds of weight and height was used to differentiate these two measures in terms of relating variables in the population. As Tables 3 and 4 in Appendix 2 show, social class gradient is apparent across the thirds of height, while such a gradient can not be seen across the thirds of weight. Taller people are also more likely to be from higher social class and shorter ones are more likely to be from lower social class. Social class gradient for height was reported by previous studies for children and adults (274, 275, 459-61). This may indicate that taller people have been grown up in better environment from childhood. Also, taller subjects were more likely to be active (defined by activity score), and be younger than shorter individuals. For smoking habits there was no gradient across the thirds of height. Across the thirds of weight there was no obvious social class gradient. However, people

with higher social class may intend to lose their weight because of health considerations. Heavier subjects were not differed from thinner individuals in terms of physical activity and long illness, but were less likely to smoke.

Based on the differences between height and weight in associations with other variables related to bone health and dietary intake, and regarding to the current knowledge of the mechanism of action on bone, allied to the debates about the using of BMI in older people, it was decided to use height and weight as measures of body and skeletal size for further analysis on diet and bone health.

6.2. Body weight and height, in NDNS

In the data of NDNS, reliable measurements of height were obtained for 1217 (629 men and 588 women) subjects and those of weight were obtained for 1319 (673 men and 646 women) subjects. Descriptive of body weight and height for dietary sample (subjects who provided a full 4-day dietary record) is presented in Table-6.2.

Table 6. 2-Body weight and height among the whole sample by sex and domicile index.

Gender		Body weight (kg)	Height (m)
Men			
Free-living	N[1]	539	521
	Mean	76.5	1.70
	Lower third	71.1	1.67
	Upper third	81.0	1.73
	Std. Deviation	12.4	0.07
Institution	N	113	88
	Mean	66.2	1.64
	Lower third	59.6	1.59
	Upper third	69.8	1.69
	Std. Deviation	13.1	0.08
Women			
Free-living	N	514	489
	Mean	65.0	1.56
	Lower third	59.7	1.53
	Upper third	69.2	1.59
	Std. Deviation	12.3	0.06
Institution	N	102	71
	Mean	56.5	1.50
	Lower third	51.3	1.46
	Upper third	61.5	1.53
	Std. Deviation	10.9	.08

N= Number of cases with valid values, reliable measures and 4-day dietary records.

Both body weight and height were normally distributed among the whole sample with a mean of 68.2 Kg (SD = 14.0) for weight and 1.6m (SD = 0.10) for height.

Initial analyses revealed high correlations of these two variables to plasma ALP and dietary scores. Table-6.3 presents the correlation coefficients between dietary scores and plasma alkaline phosphatase in one hand and body weight and height on the other. Almost all dietary patterns were related to the weight and height. These two variables were also significantly related to the plasma ALP.

Table 6. 3- Pearson's correlation coefficients between body weight and height, alkaline phosphatase and dietary patters (NDNS).

		Dietary patterns						
	Alkaline phosphatase[1]	Healthy	Traditional	Sugary-dairy	Alcoholic	vegetarian	Butter	Margarine
Men								
Weight	-0.12	0.21	0.13	-0.8	0.15	0.04	0.08	0.06
P value	0.05	0.00	0.00	0.05	0.00	0.26	0.03	0.11
Height	-0.05	0.23	0.03	0.05	0.13	0.03	0.02	0.04
P value	0.29	0.00	0.39	0.22	0.00	0.44	0.54	0.32
Women								
Weight	-0.02	0.23	0.03	-0.20	0.01	0.11	0.13	0.00
P value	0.51	0.00	0.40	0.00	0.85	0.00	0.00	0.92
Height	-0.08	0.25	0.05	-0.03	-0.01	0.16	0.03	-0.06
P value	0.05	0.00	0.21	0.41	0.84	0.00	0.46	0.11

[1]. Log transformed values

To assess the effects of body size on the relations between dietary patterns and bone health, we cross-classified the study population by sex- and domicile-specific thirds of weight and height, creating nine groups from possible combinations of height and weight. Associations between bone health and diet were analysed for each of the nine groups, separately. We used thirds in order to keep the number of groups reasonably low for the sake of simplicity of comparisons and analyses, to reduce the probability of misclassification and to lose less data due to grouping scheme.

Differences in dietary scores across the thirds of weight and height were firstly examined. As is presented in Table 6.4, the mean and confidence intervals were different across the thirds of weight and height for most dietary patterns. The differences of healthy dietary

pattern however, were more highlighted than other dietary patterns. From this table it is clear that the tallest and heaviest subjects were most likely to practice a healthy dietary pattern. It is also clear that subjects with different body size my have different dietary patterns.

However, because the amount of food eaten may affect the dietary scores and people with a bigger body size need more foods, total energy intakes should be controlled for. Using a model of ANOVA, controlling for energy intake, showed that variations of healthy diet scores were significantly predicted by height in men and by both height and weight in women, with more pronounced effect of height in both sexes (see Table 5 in Appendix 2).

Table 6. 4-Descriptive of dietary scores across thirds of weight and height.

		Weight thirds			Height thirds		
		First	Second	Third	First	Second	Third
Healthy							
Mean		-0.16	0.27	0.35	-0.07	0.24	0.49
95 % CI	Lower bound	-0.27	0.17	0.25	-0.17	0.14	0.38
	Upper bound	-0.06	0.36	0.45	-0.03	0.35	0.60
Traditional							
Mean		-0.03	0.11	0.10	-0.01	0.11	0.12
95 % CI	Lower bound	-0.13	0.02	0.00	-0.11	0.01	0.02
	Upper bound	0.07	0.20	0.19	0.08	0.201	0.21
Sugary-dairy							
Mean		0.02	-0.12	-0.26	-0.16	-0.25	0.12
95 % CI	Lower bound	-0.07	-0.21	-0.35	-0.26	-0.34	-0.21
	Upper bound	0.11	-0.03	-0.17	-0.07	-0.16	-0.03
Alcohol-trend							
Mean		-0.10	0.01	0.06	-0.17	0.10	0.07
95 % CI	Lower bound	-0.19	-0.08	-0.06	-0.27	0.00	-0.05
	Upper bound	-0.01	0.10	0.17	-0.08	0.20	0.19
Veg-trend							
Mean		-0.06	0.06	0.06	-0.07	0.02	0.14
95 % CI	Lower bound	-0.15	-0.04	-0.04	-0.17	-0.09	0.04
	Upper bound	0.03	0.16	0.16	0.02	0.12	0.23
Pattern 6							
Mean		-0.08	0.01	0.19	0.04	-0.01	0.07
95 % CI	Lower bound	-0.18	-0.08	0.10	0-.06	-0.10	-0.03
	Upper bound	0.02	0.11	0.28	0.13	0.09	0.16
Pattern 7							
Mean		-0.09	0.04	-0.03	-0.01	-0.09	-0.07
95 % CI	Lower bound	-0.18	-0.06	-0.12	-0.11	-0.19	-0.17
	Upper bound	0.01	0.15	0.07	0.09	0.01	0.03

Using a model of ANOVA (with log-transformed values of ALP as dependent and thirds of weight and height as predictors), weight was significantly associated with plasma ALP in men, while, neither height nor weight could significantly predict plasma ALP in women (see Table 6 in Appendix 2).

Variation of dietary scores and ALP across the groups of weight and height were also examined in men and women, separately. Figures 6.1-4 present the distribution of healthy diet score and ALP across the weight/height groups in men and women, separately.

Among women for each third of weight, a positive trend is obvious across the thirds of height and vice versa. Similar trend is obvious for men except among those within the lowest third of height. Heavy tall (HT) subjects had significantly higher scores on healthy dietary pattern in comparison with the thin-short (TS) people in both sexes. Controlling for total energy intake did not change this trend. As is shown in Figures 6.1 and 6.2, interaction between body size and dietary patterns varied by sex but generally short people had lower dietary scores on healthy diet in comparison to the other groups. In men, heavy-short individuals had the most negative trend toward the healthy diet. It means that stunting heavy men consumed more from sugar and puddings and less from fruits and vegetables. In women, on the other hand, the most negative trend toward healthy diet was seen in thin-short women. Stunted heavy women had positive scores, even though had lower scores than heavy-tall women. Heavy-tall people from both sexes consumed more healthy foods even after controlling for energy intake. Controlling for BMI, energy intake and social class, which were found to be related to healthy diet score, did not change the trend substantially, although differences between some weight and height groups disappeared (see Figures 1 and 2 in Appendix2). It should be borne in mind that positive score on healthy diet indicates both higher trend toward healthy foods and negative trend toward unhealthy foods, with magnitude of the score indicating how likely is for a person to eat healthy foods in comparison to others. Given the associations between bone health and healthy diet (and in general the association between diet and health), theses results may be of particular importance for public health nutrition professionals to identify higher risk groups in the population who may become the target of special interventions.

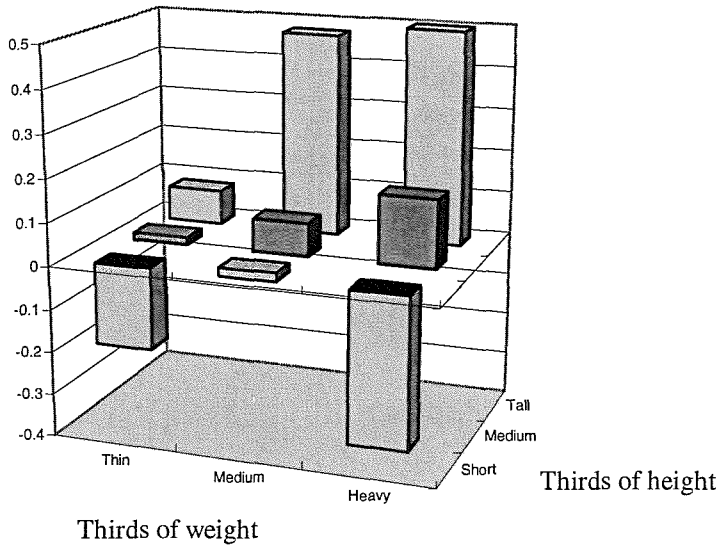


Figure 6. 1-The distribution of healthy diet scores among men across thirds of weight and height (NDNS). Bar charts represent the mean of healthy diet score in each group.

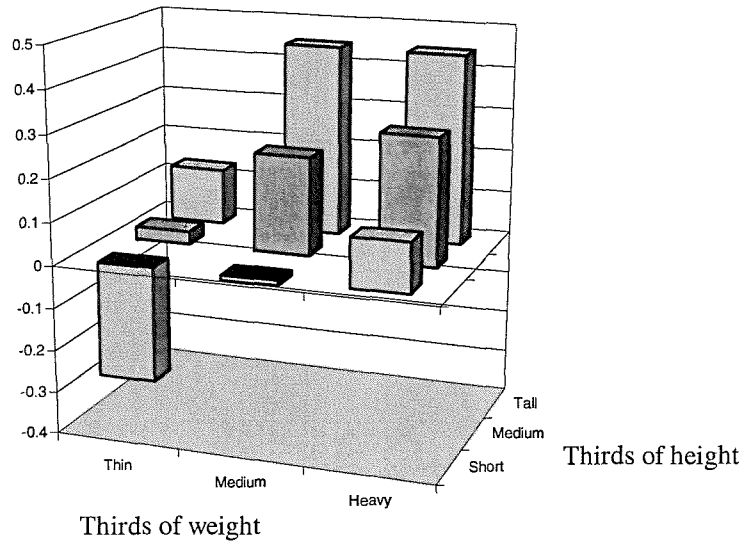


Figure 6. 2- The distribution of healthy diet scores among women across thirds of weight and height (NDNS). Bar charts represent the mean of healthy diet score in each group.

Differences between groups for ALP however, were not considerable. Using one-way ANOVA, although for the entire sample and for free-living people, variation of ALP across the groups was significant, in women and institution people it was not significant.

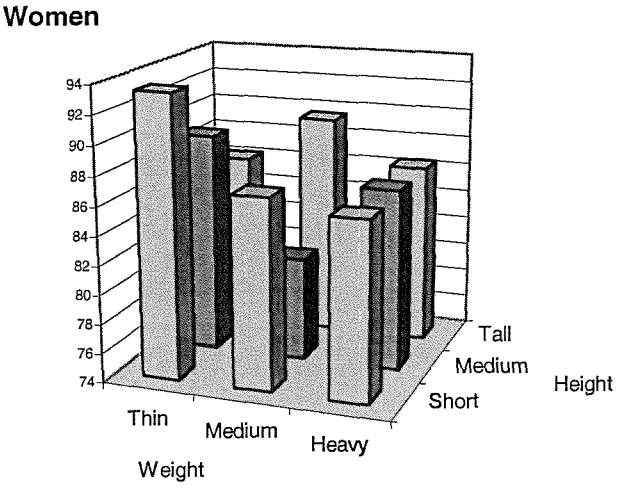


Figure 6. 3- Distribution of Plasma ALP by thirds of weight and height in women (NDNS). Bar charts represent the mean of healthy diet score in each group.

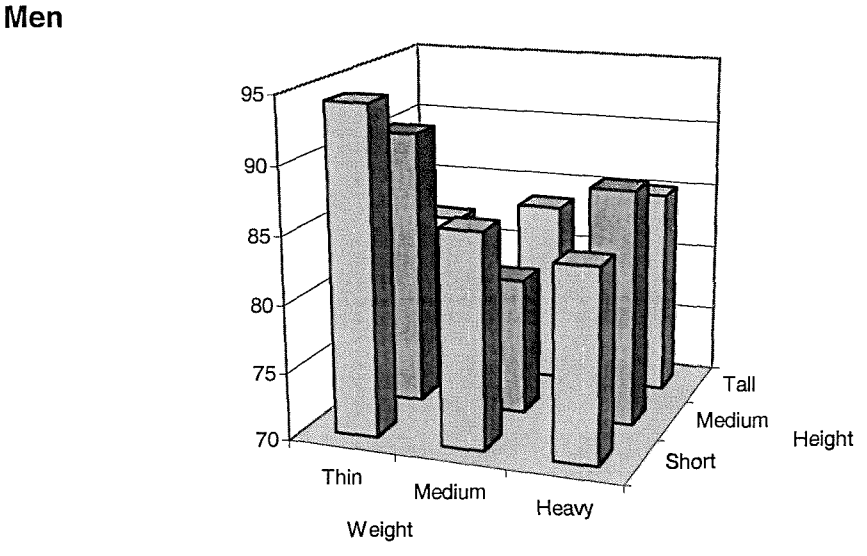


Figure 6. 4- The distribution of ALP by thirds of weight and height among men (NDNS). Bar charts represent the mean of healthy diet score in each group.

Among men, differences between groups in term of ALP were significant ($F_{(8, 388)}$ =2.3, P =0.02), which was mostly due to differences between short-thin group and medium group (Figure 6.3). These two groups were also significantly different in women ($t_{(df=148)}$ = -2.6, P = 0.04). However, in both sexes the short-thin subjects had the highest level of ALP and the people with the medium thirds of height and weight had the lowest plasma level of ALP.

Relationships between dietary patterns and plasma alkaline phosphatase across the groups were examined by Pearson's correlation coefficients in men and women, separately. Table 6.5 presents these coefficients for four groups, which are at the extreme thirds of weight and height for men and Table 6.6. presents the same correlation matrix among women. Note that the value of plasma alkaline phosphatase, dietary vitamins and dietary copper were $\log_e$ transformed to achieve normality.

Table 6. 5- Pearson's Correlation coefficients between ALP and dietary variables across weight and height groups, men[1] (NDNS).

	TS (n=84)		TT (n=20)		HS(n = 23)		HT (n=88)	
% of free-living	72%		79%		75%		90%	
	r	*P*	r	*P*	r	*P*	r	*P*
Vitamin A	0.06	0.61	0.32	0.18	0.22	0.33	-0.16	0.17
Vitamin C	0.01	0.96	-0.02	0.94	0.17	0.47	-0.20	0.09
Vitamin E	-0.10	0.40	0.23	0.35	0.42	0.06	-0.20	0.09
Vitamin D	-0.20	0.09	0.01	0.97	0.41	0.07	0.08	0.52
Zinc	-0.17	0.15	-0.08	0.74	0.35	0.11	-0.18	0.13
Copper	-0.01	0.95	-0.07	0.76	0.19	0.40	-0.14	0.24
Magnesium	-0.17	0.15	-0.12	0.61	0.12	0.60	-0.29	0.01
Calcium	0.01	0.93	0.09	0.71	0.43	0.05	-0.06	0.63
Phosphorus	-0.12	0.33	-0.04	0.88	0.32	0.16	-0.26	0.02
Potassium	-0.20	0.09	0.07	0.77	0.24	0.30	-0.27	0.02
Protein	-0.22	0.06	0.18	0.47	0.23	0.32	-0.16	0.17
Healthy	-0.09	0.46	-0.35	0.15	0.14	0.55	-0.43	0.00
Traditional	-0.06	0.60	0.28	0.25	-0.01	0.97	0.12	0.29
Sugary-dairy	0.35	0.00	0.06	0.81	0.41	0.06	-0.02	0.85
Alcohol-trend	-0.28	0.02	-0.13	0.59	-0.16	0.48	-0.29	0.01
Veg-trend	-0.21	0.08	0.00	0.99	-0.26	0.25	0.12	0.32
Pattern 6	-0.13	0.28	0.18	0.47	0.06	0.80	0.22	0.06
Pattern 7	0.15	0.20	-0.13	0.61	0.35	0.12	0.15	0.19

[1]Pvalues are 2-tailed.

The pattern of relationships between alkaline phosphatase and dietary variables varied greatly in men and women. While, the healthy dietary pattern in men of the heavy-tall

group was highly correlated with ALP, in corresponding group of women it was not related to ALP.

Across the presented groups in Table 6.5 correlations between dietary variables and alkaline phosphatase were mostly significant for heavy-tall group (HT) of men and the highest correlation for healthy dietary pattern was also seen in this group. Negative sign of this correlation coefficient indicates the positive association between healthy dietary pattern and bone health. Among men in thin-short and heavy-short groups the third dietary pattern (sugary-dairy diet) was positively related to alkaline phosphatase (negative association with bone health).

Among women on the other hand, dietary variables were not related to alkaline phosphatase in almost all groups, except among the heavy-short people, in whom minerals and vitamin E were related to ALP, significantly.

Table 6. 6-Pearson's Correlation coefficients between ALP and dietary variables across weight and height groups, women[2] (NDNS).

	TS (n=67)		TT (n=22)		HS(n = 31)		HT (n=70)	
% of free-living	72%		90%		90%		92%	
	r	*P*	r	*P*	r	*P*	r	*P*
Vitamin A	0.02	0.90	0.13	0.62	-0.20	0.38	0.17	0.22
Vitamin C	-0.23	0.08	0.33	0.19	0.10	0.67	0.06	0.66
Vitamin E	-0.16	0.21	-0.15	0.58	-0.50	0.02	-0.35	0.01
Vitamin D	-0.07	0.56	-0.20	0.44	-0.09	0.70	-0.23	0.09
Zinc	-0.06	0.63	0.05	0.86	-0.45	0.04	-0.19	0.17
Copper	0.03	0.81	0.52	0.03	-0.33	0.14	-0.08	0.57
Magnesium	0.03	0.84	0.32	0.21	-0.54	0.01	-0.05	0.70
Calcium	0.17	0.17	-0.24	0.35	-0.48	0.03	0.09	0.52
Phosphorus	0.13	0.32	0.12	0.65	-0.56	0.01	0.00	1.00
Potassium	0.04	0.78	0.24	0.36	-0.45	0.04	-0.06	0.67
Protein	0.04	0.74	0.02	0.95	-0.42	0.06	-0.17	0.21
Healthy	-0.06	0.64	0.01	0.96	0.13	0.58	-0.07	0.62
Traditional	0.20	0.12	-0.07	0.80	0.14	0.55	-0.15	0.28
Sugary-dairy	-0.16	0.22	0.19	0.46	-0.24	0.30	0.04	0.78
Alcohol-trend	-0.13	0.30	0.46	0.06	0.09	0.71	-0.13	0.35
Veg-trend	-0.22	0.08	0.19	0.47	0.04	0.88	0.18	0.18
Pattern 6	-0.18	0.16	-0.18	0.50	-0.45	0.04	-0.36	0.01
Pattern 7	-0.04	0.77	0.49	0.04	-0.08	0.74	-0.01	0.96

2.Numbers represent the correlation coefficients (r) and *P* values are two tailed.

Great modification effects of sex and body size are apparent from these two tables. Huge differences between men and women in the pattern of relationships between diet and bone health may indicate the effect of sex hormones or difference in the nature of bone metabo-

lism between men and women. These data suggest that a diet rich of healthy foods may be not necessarily healthy for all individuals in a population, and the negative association between some food groups such as sugary foods and bone health may be limited to heavy persons with short stature.

From these results it is obvious that the relationship between dietary patterns and alkaline phosphatase is remarkably different in men and women with weaker associations in women than in men. Also, the relationships between dietary variables and bone health are more highlighted in heavy and tall people than did in all other subjects in the population.

To explore the association between dietary patterns and bone health and accounting for other bone affecting variables, a model of multiple regression analysis was used. The same model of multiple regression analysis as did in chapter five was performed for each group of height and weigh, independently. Again logarithmically transformed values of plasma alkaline phosphatase was used as dependent variable and dietary scores on seven identified dietary patterns, energy intake and a set of dummy variables for domicile, long-standing illness, physical activity, smoking habits, malnutrition risk and age were entered as independent variables and the method of stepwise multiple regression analysis was used similarly for all weight and height groups. All analyses were undertaken separately for men and women.

The results of multiple regression analysis across different groups of weight and height are presented in Table 6.7 and 6.8. Among men within the group of HT healthy dietary pattern was the only predictor of bone health ($B = -0.03$, $R^2 = 0.19$, $F_{(1,71)} = 16.7$, $P=0.000$) and other variables were excluded from the analysis because of non-significant effect. Among this group of subjects healthy dietary pattern was accounted for 19% of variation in alkaline phosphatase. The results for other weight and height groups were different. Healthy diet predicted bone health in two groups out of nine. The greatest amount of variance in ALP was explained by healthy diet among subjects within the group of Heavy tall. Heavy subjects with moderate stature did also benefit from "healthy diet". "Traditional meat-trend diet" and "sugary-dairy diet" were also negatively related to bone health in men who were medium and tall and those who were thin and short, respectively.

Table 6. 7- Stepwise Multiple regression analysis[1] for men across the groups of weight and height: Partial regression coefficients (B), standardized regression coefficients (β), change in R^2 (ΔR^2) and statistical significance. Dependent: ALP, independents, seven dietary scores, energy intake, domicile, smoking, malnutrition risk, activity, long illness (NDNS).

Group	Variables	B	β	R^2	Adjusted R^2	ΔR^2	*P* value
Heavy-tall (HT)	Healthy diet	-0.03	-0.44	0.19	0.18	0.19	0.00
Heavy-medium (HM)	Healthy diet	-0.04	-0.31	0.10	0.08	0.10	0.03
Heavy-short (HS)	-	-	-	-	-	-	-
Medium-tall (MT)	Traditional diet[2]	0.04	0.32	0.10	0.09	0.10	0.02
Medium-medium (MM)		-	-	-	-	-	-
Medium-short (MS)	-	-	-	-	-	-	-
Thin –tall (TT)	-	-	-	-	-	-	-
Thin-medium (TM)	-	-	-	-	-	-	-
Thin-short (TS)	Sugary-dairy	0.04	0.35	0.13	0.11	0.13	0.00

[1]Only dietary patterns, with a significant effect are presented.

[2]Traditional meat-trend diet.

Table 6.8 presents the results of multiple regression analysis among women. The "healthy diet" did not predict ALP in any of the weight and height groups. Among heavy-tall females only the sixth dietary pattern was related to ALP, in a negative way, indicating a positive association with bone health. Rather similar associations were found in heavy-short women. In medium-short people alcohol-trend diet negatively associated with bone health. These results suggest that females with different body size may deal with the same food in a variable way, and, therefore, an eating pattern which seems to be beneficial for some people may not be necessarily of benefit for others, with different body size.

Table 6. 8-Stepwise Multiple regression analysis* for women across the groups of weight and height: Partial regression coefficients (B), standardized regression coefficients (β), change in R^2 (ΔR^2) and statistical significance. Dependent: ALP, independents, seven dietary scores, energy intake, domicile, smoking, malnutrition risk, age, activity, long illness (NDNS).

Group	Dietary Variables	B	β	R^2	Adjusted R^2	ΔR^2	*P* value
Heavy-tall (HT)	Butter	-0.06	-0.39	0.15	0.14	0.15	0.00
Heavy-medium (HM)	-	-	-	-	-	-	-
Heavy-short (HS)	Butter	-0.11	-0.62	0.39	0.36	0.39	0.00
Medium-tall (MT)	-	-	-	-	-	-	-
Medium-medium (MM)	-	-	-	-	-	-	-
Medium-short (MS)	Alcohol-trend diet†	0.07	0.31	0.63	0.60	0.10	0.00
Thin –tall (TT)	-	-	-	-	-	-	-
Thin-medium (TM)	-	-	-	-	-	-	-
Thin-short (TS)	-	-	-	-	-	-	-

*Only dietary patterns, with a significant effect are presented.
† The alcohol-trend diet entered the model after malnutrition risk and physical activity..

6.3. Summary

Analyses presented in this chapter were concerned about the objective of the second hypothesis, to see how body size may affect associations between eaten foods and bone health. From the previous chapters it was concluded that dietary patterns may be more related to ALP than isolated nutrients. It was also concluded that the "healthy diet" may be associated with better bone health in men and women, in general. In this chapter, stratified analyses for different body sizes showed that the size of the body may modify the association between diet and ALP in both sexes. The results again proved that a diet, which may seem to benefit bone health in a subgroup of the population, may not benefit others or may be even harmful for others and body size may be a key determinant in this respect. In some groups of body size, none of dietary patterns could predict ALP, suggesting the priority of other variables in comparison with dietary factors in relation to ALP and bone health. However, bone health is influenced by many factors other than diet such as heredity and environment.

Presented analyses in this chapter showed that body size is associated with the pattern of food consumption, even after controlling for BMI, energy intake and socioeconomic class. Short heavy men and short thin women were less likely to eat healthy foods and were more likely to eat sugar, pudding and unhealthy foods. These results agree with those found in other studies that heavy-stunted people are consuming more from unhealthy foods (411;464). However, others reported that eating behaviour in these people is associated with BMI. What is added by the present analyses is that following an eating behaviour may be beyond BMI. Adjusting for BMI did not remove the differences in eating patterns among different w/h groups. It may suggest the priority of weight and height for representing eating behaviours in the population.

There is convincing evidence that people from higher socio-economic groups eat more healthy than others from lower classes (465-468) but in the present analysis, controlling for socioeconomic class did not remove the differences between w/h groups (Figures 1 and 2 in Appendix 2). However, controlling for social class may not account for all variables, which may affect eating behaviours in different social groups.

However, controlling for BMI, energy intake and social class, using ANOVA, revealed a negative trend among short men toward healthy diet by increasing the weight, while such a trend disappeared in tall men. In short women, increasing the weight (by thirds) accompanied by an increase in healthy diet score. In tall females, like men, no trend was obvious across the thirds of weight (Figure 2 in Appendix 2).

Association between body size and dietary patterns may be in some part related to some other unrecognised variables, for which weight and height may be representative. Although differences in healthy dietary score by body size explained in some part by social class and fatness (BMI), adjusting for these two variables and for the total energy intake, to eliminate the effect the amount of eaten foods, did not remove the differences between the groups for their trend toward healthy diet. However, dietary patterns may be a consequence of cultural and ethnic heritage and of many environmental factors, including the availability of foods, the ability to purchase and prepare foods, the numerous advertisements for foods and taste preference (454) and many of these factors may be in some way represented by body size.

In some part, eating behaviour is influenced by genetic factors (443;444;469-71). Using pattern analysis in a twin study, Van den et al (470) reported that 33-40% of variance of a healthy dietary pattern, which was somewhat similar to the healthy diet in the present study, explained by genetic factors. Similar figures reported by others for meal size, energy intake and macronutrient intakes (443;444;469;471), albeit others reported no genetic influence (472). Association between dietary pattern and body size may be, in some part, determined by similar genetic component, by which both food intake and body size are influenced.

The results of the present study suggest that the relationships between diet and bone health is influenced by body size and gender. Among taller and heavier men, the association between bone health and healthy diet is stronger than that of shorter and thinner people. This study gave a unique picture of the modification effect of body size on the relation between dietary patterns and bone health. To my knowledge this the first study, in which by the use of stratified analysis such effect of body size is reported. Positive associations between body size and bone health was reported previously and several underlying mechanisms have been proposed, mostly from investigations on the relations between body height and chronic diseases, such as CHD, diabetes and cancer (see chapter 2 and the beginning of this chapter). But whether this positive association is because of more benefit from healthy foods or is because of other factors for which body size is representative, has not been addressed in previous studies. However, presented results suggested that in some part, associations between body size and bone health may be due to its effects on the relations between diet and bone health. In this respect, if the results of this study are valid people with different body sizes will benefit from foods differently. Likewise, their nutritional demands are different and should be satisfied differently. Therefore, it may not be proper to advise them in the same way. However, in interpretation of the results of this study two important points should be borne in mind; firstly, because it was a cross-sectional study, drawing causal inferences from the data is limited and therefore obtained associations should be interpreted with caution. Secondly, w/h groups were different in terms of some variables such as smoking, age, socio-economic class, physical activity, malnutrition risk and proportion of free-living and institutionalised subjects,

which were taken to account (see Appendix 2, Table 7). There may be other unaccounted variables, which could partly explain obtained results, such as sunshine score and years since menopause. Furthermore, proportion of those using weight control diets together with change in lifestyle may be more in heavy-tall people, due to higher social class in this group, and may have affected the results. These people could not be recognised from NDNS data and may lead to an overestimate the effect of healthy diet. They may also report a healthier diet, which may not be excluded by excluding under reporters. However, all analyses were controlled for extraordinary situations that may have affected the results, such as subjects who suffering from morbid diseases i.e. cancer, kidney and endocrine diseases or situations, which may directly affect ALP, such as hepatic diseases and kidney dysfunction. Excluding those with outlier values of ALP and those who suspected to suffer from liver diseases (based on plasma GGT) and those taking thiazide antidiuretic, anticonvulsants and HRT, it is unlikely that some situations such as fatty liver or cholestasis, which is more common in obese subjects have affected the results. Saving the space, possible explanations for the effect of body size and gender is presented in Chapter 8, which is dedicated to the overall discussion.

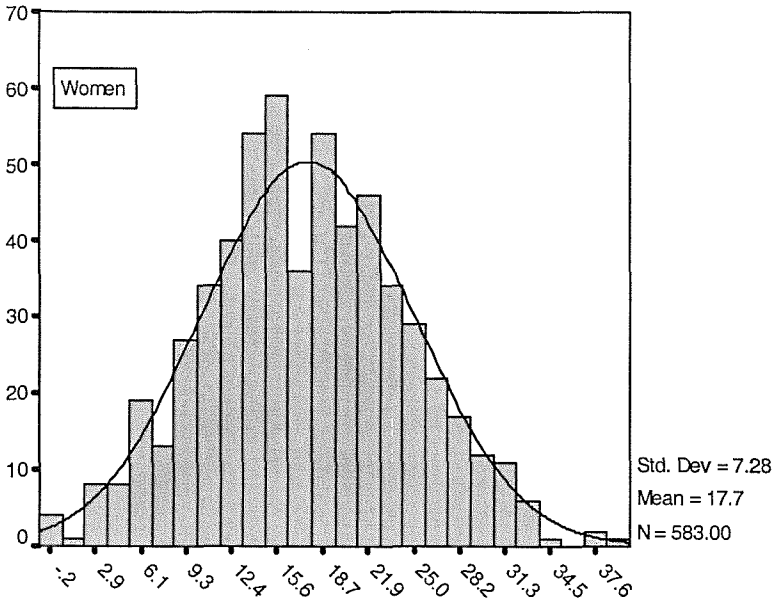
7. Eating patterns and Handgrip strength

As has so far been described in chapter two, fall is among the major causes of mortality and morbidity in the elderly. It is also a principal determinant for osteoporotic fractures as, for example, more than 90% of osteoporotic fractures are due to a fall (363;364). Several intrinsic and extrinsic factors may contribute the risk of fall in the elderly. The most important intrinsic factor leading to a fall is muscle weakness (368) (see chapter two). To date, there is little evidence from observational studies linking muscle weakness to dietary variables, although in many studies muscle performance tests have been used as markers of general nutritional state. In this chapter, the relationship between muscle function and dietary patterns will be investigated, using a marker of muscle function, namely, handgrip strength, which is a measure of muscle strength, and has been shown to be related to the risk of falling in the elderly (370). This chapter concerns the third aim of the study; to explore whether dietary patterns are important determinants of muscle weakness in the elderly.

7.1. Variation of Handgrip strength by other variables

From NDNS reliable measures of handgrip strength was available for 1248 persons in the dietary sample (those who completed a full 4-day dietary record), from them 36 were excluded because of using musculoskeletal medicines. Techniques of grip strength measurement have been described in section 3.2.3 (see page 146). Handgrip strength was normally distributed with the mean of 24.1 kg (SD =12.4 Kg) for the entire sample (Figure 7.1). Generally, the mean handgrip strength of men was significantly greater than that of women (33.2 Kg vs. 17.9Kg). Free-living people had higher values than institution people (26.8 Kg vs.13.1Kg).

Pearson's correlation coefficients were used to establish the univariate relationships between handgrip strength with body weight, stature, mid-arm circumference, BMI and dietary variables in men and women, separately.



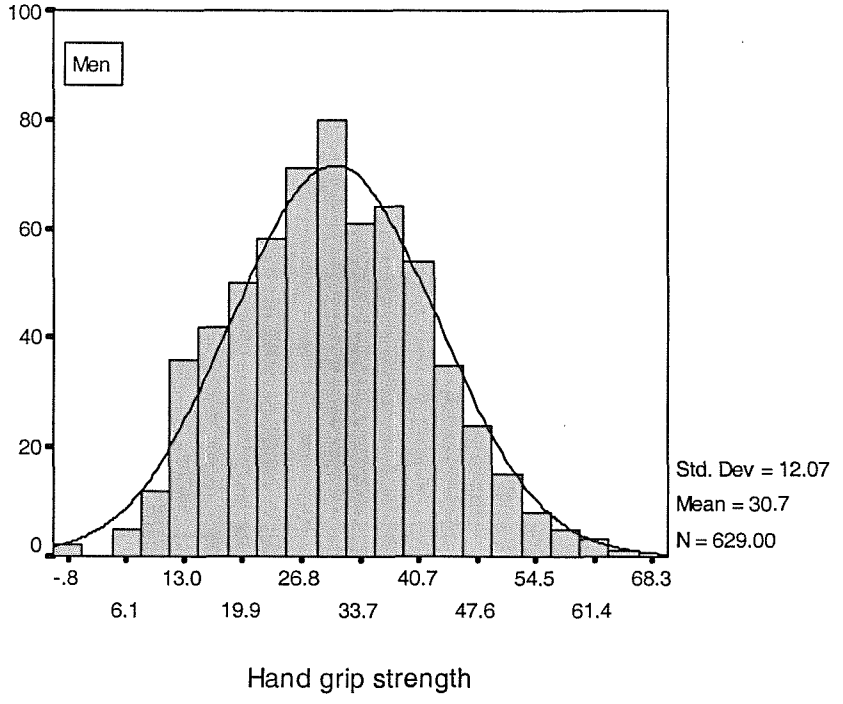


Figure 7. 1- Distribution of hand grip strength in men and women (NDNS).

Table 7.1 presents the correlation coefficients of handgrip strength with other anthropometric measures and their statistical significance. As presented in this table, associations between handgrip strength and other anthropometric measures were more stronger in men than in women. In both sexes, handgrip strength was more closely associated with height than with weight. This implied that there may be a closer association of overall skeletal size rather than body mass or muscle mass. Body mass index however was not related to handgrip strength in women, while it was still significantly associated with handgrip strength in men, even though with smaller correlation coefficients than other anthropometric measures.

Table 7. 1 - Pearson's correlation coefficients between hand grip strength with mid-upper arm circumference (MUAC) and body size measures in men and women[1] (NDNS).

	Hand grip strength			
	Men (n = 629)		**Women** (n = 583)	
	r	*P* value	r	*P* value
MUAC	0.43	0.00	0.18	0.00
Weight	0.38	0.00	0.21	0.00
Height	0.41	0.00	0.36	0.00
Body mass indexı	0.19	0.00	0.04	0.52
Age	-0.46	0.00	-0.44	0.00

1 All *P* values are two-tailed.

Variation of handgrip strength by age, the risk of malnutrition, smoking, physical activity, domicile and longstanding illness was also examined. As shown in Figure 7.2 and 7.3, handgrip strength declined significantly by age in both sexes. From the age of 65-y to the age of >85 y women lost about 42.5% of their grip strength and men lost about 41.1%. Likewise, inactivity and longstanding illness were associated with weaker grip strength in both sexes. However, the risk of malnutrition in women was not significantly associated with handgrip strength, while in men malnutrition risk was associated with a significant decline in grip strength. Smoking in both sexes was not related to the grip strength.

7.2. Eating patterns and "Handgrip strength"

As shown in Table 7.2 grip strength was positively correlated with dietary variables in both men and women. The strongest association was seen for the "healthy diet" in both sexes. Other dietary patterns were also related to handgrip strength in both men and women with rather smaller correlation coefficients.

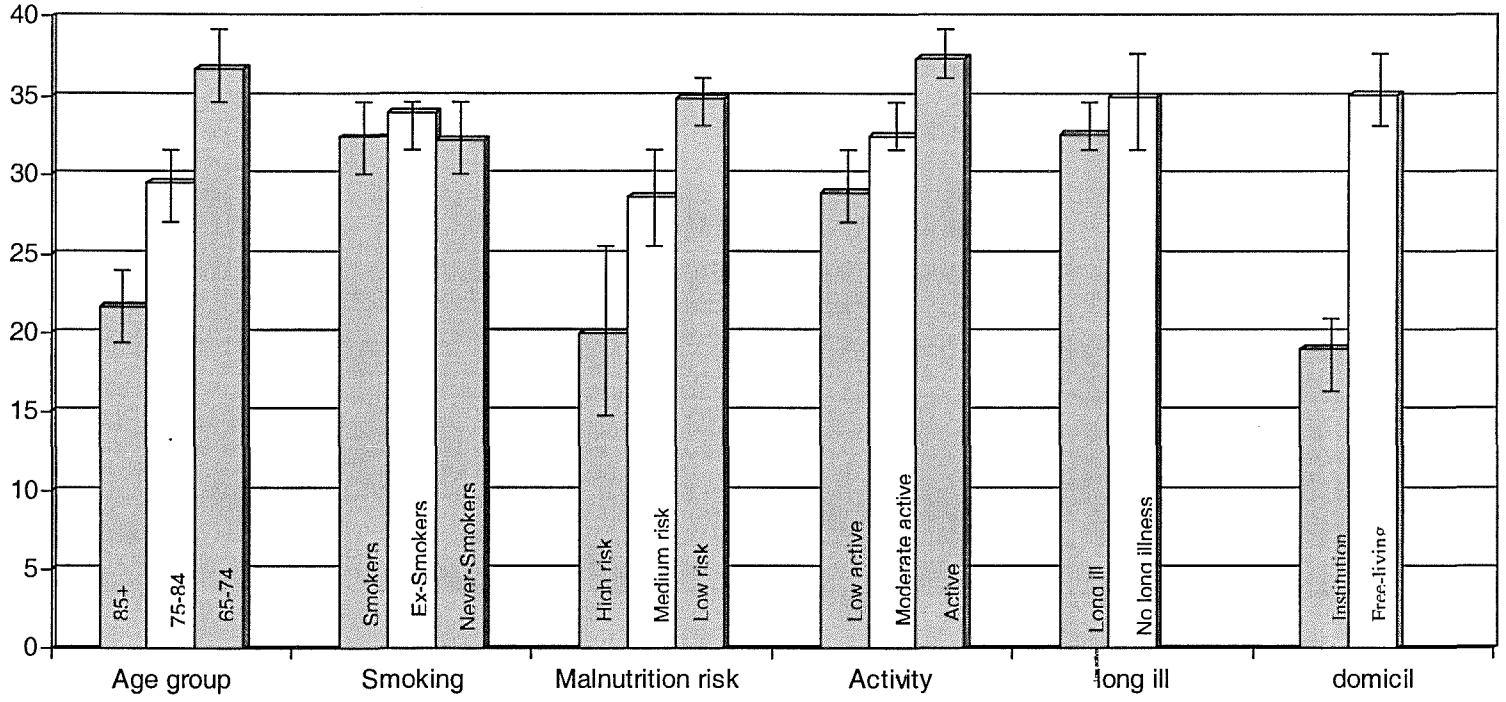


Figure 7. 2-Distribution of hand grip strength by background and lifestyle variables among men (mean and 95%CI), NDNS.

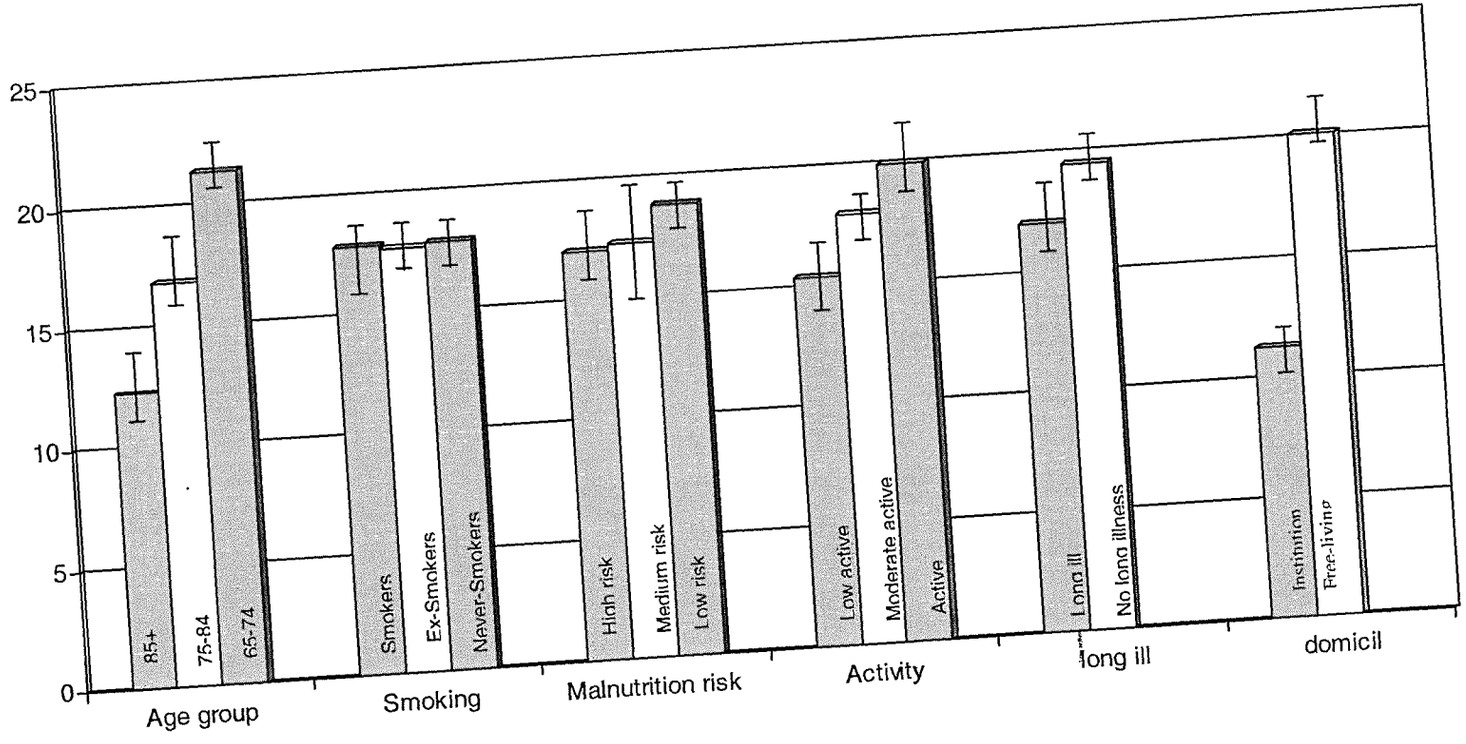


Figure 7. 3-Distribution of hand grip strength by background and lifestyle variables among women (mean and 95%CI), NDNS.

In both sexes, "sugary and dairy diet", characterised by high intakes of canned fruits, sweet foods and dairy products, was negatively associated with handgrip strength. The relationships between dietary factors and grip strength were not similar in men and women. Generally, in men these associations were stronger than those of women. Such correlations are difficult to interpret but the modification effect of sex, which is probably because of sex hormone profiles, is apparent.

Table 7. 2- Pearson's correlation coefficients between dietary variables and grip strength in men and women[1] (NDNS).

Dietary variables	Hand grip strength			
	Men (n = 490)		Women (n = 590)	
	r	*P* value	r	*P* value
Vitamin D	0.05	0.18	-0.15	0.00
Magnesium	0.28	0.00	0.15	0.00
Calcium	0.09	0.02	-0.06	0.09
Phosphorus	0.20	0.00	0.02	0.57
Potassium	0.29	0.00	0.17	0.00
Protein	0.23	0.00	0.04	0.23
Total energy	0.17	0.00	-0.09	0.01
Healthy diet	0.31	0.00	0.34	0.00
Traditional	0.15	0.00	-0.04	0.26
Sugary-dairy	-0.11	0.01	-0.22	0.00
Alcohol-trend	0.13	0.00	-0.08	0.03
Veg-trend	0.01	0.72	0.12	0.00
Pattern 6	0.11	0.01	-0.06	0.09
Pattern 7	-0.03	0.42	-0.09	0.02

1 All *P* values are two-tailed and vitamin D is $\log_e$ transformed.

In interpretation of these results, it is important to note that correlation does not imply causation. From these results one can not conclude whether, for example, the "sugary-dairy diet" leads to weaker grip strength or other way around. It is may be possible that people with poorer performance are more interested on this type of diet or because of their situation they have to use "easy foods" more than others who are of better performance.

7.3. Multiple regression analysis

In order to explore the relationship between dietary patterns and handgrip strength, step-wise multiple regression analysis was carried out with handgrip strength as dependent variable and dietary scores, longstanding illness, risk of malnutrition and domicile as in-dependent variables, controlling for energy intake, age, mid-upper arm circumference and body size (weight and height). Categorical data were converted to dummy variables (di-chotomous variables with the value of 1 or zero) to enter the model.

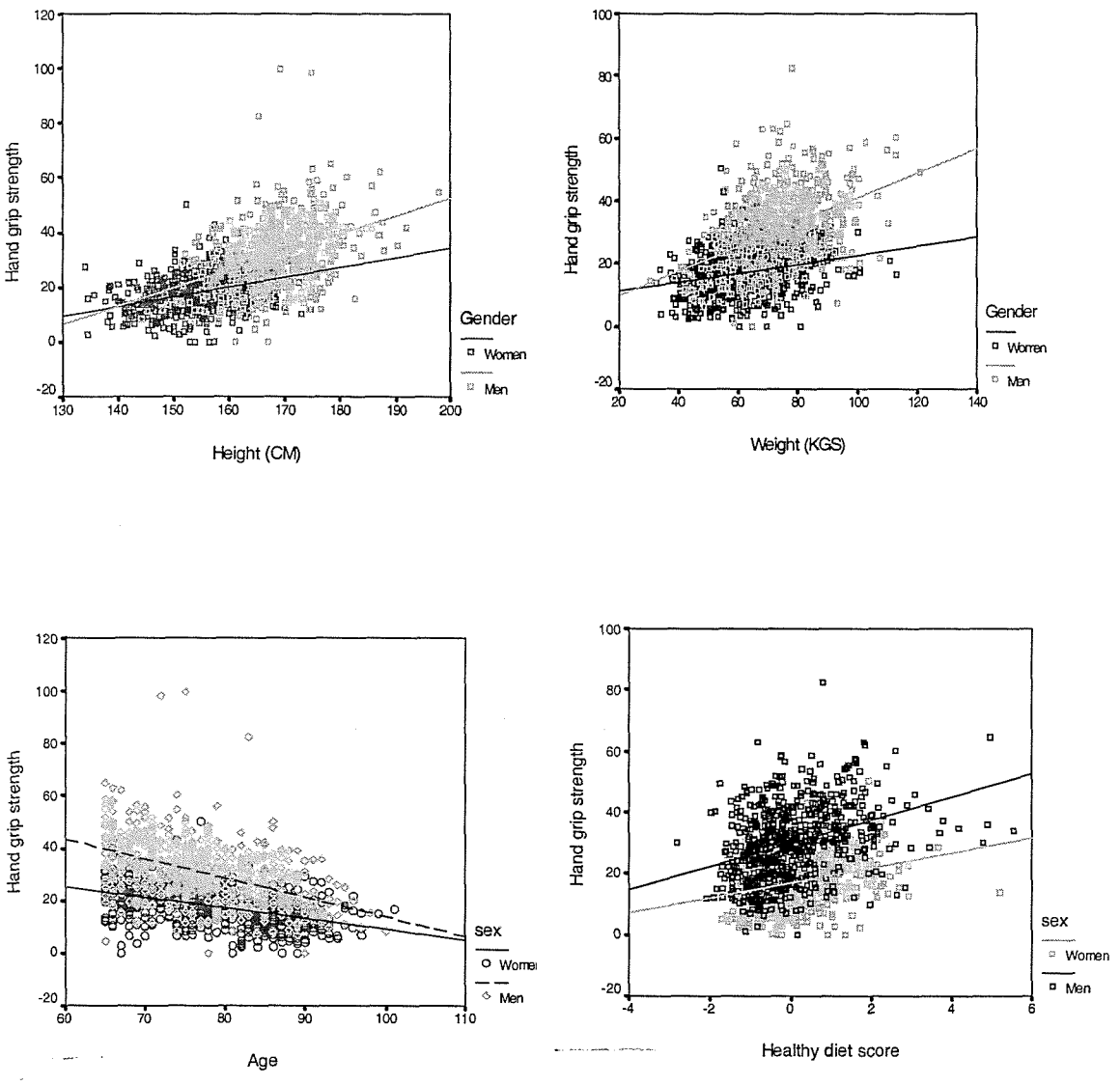


Figure 7. 4-Scatter plot of handgrip strength of males and females by weight, height, age and healthy diet score (NDNS).

Variables which did not make a significant contribution to handgrip strength were dropped. A 5% level of probability was used to indicate statistical significance. Scatter plots were produced to determine whether a linear model is reasonable for these variables. The patterns of diagrams suggested that the relationship of handgrip strength with each of predictors was linear (Figure 7.4). Plots of residual distribution confirmed that the assumptions of the analysis (linearity of relations and homogeneity of variance) have been met. Analyses were undertaken for men and women, separately.

As shown in Table 7.3, having controlled for body size, energy intake, age and possible confounders, "healthy eating pattern" could predict handgrip strength in free-living people in both sexes. Although the contribution of dietary variables in the variance of grip strength was small (see ΔR^2), it was highly significant. Among men from institution sample "alcohol-trend diet" was the only dietary pattern could predict handgrip strength. In institution women none of dietary patterns was associated with handgrip strength. However, in women weight, age and physical activity were more important than dietary scores in explaining the grip strength. In men, on the other hand, height, age, physical activity and mid-upper arm circumference appeared prior to the dietary scores for predicting grip strength. BMI dropped out of the model because of non-significant effect in both sexes.

Table 7. 3-Stepwise Multiple regression analysis* for men and women across the domicile groups: Partial regression coefficients (B), standardized regression coefficients (β), change in R^2 (ΔR^2) degrees of freedom and statistical significance. Dependent: handgrip strength, independents, seven dietary scores, energy intake, malnutrition risk, activity, long illness, age, MUAC, weight and height (NDNS).

Group	Variables	B	β	R^2	Adjusted R^2	ΔR^2	df	*P* value
Free-living								
Men	Healthy diet	1.29	0.14	0.28	0.27	0.05	422	0.00
Women	Healthy diet	0.77	0.11	0.14	0.13	0.01	516	0.01
Institution								
Men	Alcohol-trend diet	3.6	0.42	0.14	0.12	0.14	36	0.02
Women	-	-	-	-	-	-		-

*Only dietary patterns, with a significant effect are presented.

To explore non-linear components, squares of age weight and height were entered the model simultaneously with their ordinary values. All squares failed to contribute to the

model, suggesting a linear relationship between handgrip strength and both age and body size measures.

Variation of handgrip strength by season was examined using one way ANOVA. No statistically significant difference in grip strength was noted between seasons among the entire sample and both sexes, when analysed separately (Figure 7.5).

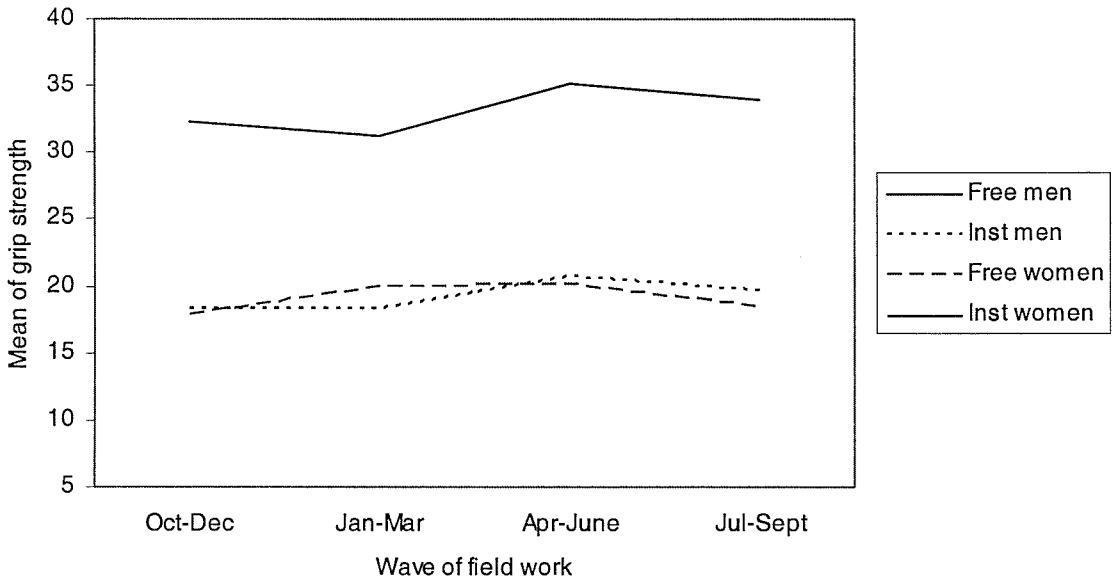


Figure 7. 5- Seasonal variation of handgrip strength by sex and domicile (NDNS).

To examine whether under-reporting may affect the results of the association between diet and handgrip strength, the model of multiple regression analysis repeated with excluding subjects with EI/BMR ratio less than 1.2. healthy dietary pattern was still a significant predictor of handgrip strength in free-living men and women after controlling for body size, energy intake, other dietary scores, malnutrition risk, activity, long illness, age and MUAC (Table 7.4).

Similarly, in institution sample the former results repeated with no substantial changes. Among institutionalised women, however, the seventh dietary pattern remined in the model because of significant association with handgrip strength. These results suggest that

it is unlikely that under-reporting of dietary intakes have biased my results of the association between dietary patterns and handgrip strength.

Table 7. 4- Stepwise Multiple regression analysis* for men and women across the domicile groups: Partial regression coefficients (B), standardized regression coefficients (β), change in R^2 (ΔR^2) degrees of freedom and statistical significance. Dependent: handgrip strength, independents, seven dietary scores, energy intake, malnutrition risk, activity, long illness, age, MUAC, weight and height (NDNS).

Group	Variables	B	β	R^2	Adjusted R^2	ΔR^2	df	*P* value
Free-living								
Men	Healthy diet	1.47	0.18	0.19	0.18	0.03	245	0.003
Women	Healthy diet	1.3	0.18	0.15	0.13	0.03	201	0.008
Institution								
Men	Alcohol-trend diet	3.3	0.42	0.17	0.14	0.17	25	0.028
Women	Seventh dietary pattern	-2.15	-0.27	0.07	0.06	0.07	51	0.047

*Only dietary patterns, with a significant effect are presented

7.4. Summary

Analyses presented in this chapter were aimed to examine the relationship between dietary patterns and handgrip strength. This chapter was devoted to the fourth objective of the study. Controlling for body size, energy intakes and a number of confounders, dietary patterns could still predict handgrip strength in both sexes. However, contribution of diet in variation of grip strength was not remarkable. In this regard, weight, height and mid-upper arm circumference, which are the measures of body size and muscle mass, were much stronger than nutritional factors. Age was also an important predictor of grip strength. Its associations were stronger than that of dietary patterns. These results may indicate that grip strength may be a measure of function (physiology) rather than metabolism, and therefore, it is determined predominantly by body build and muscle mass. It is noteworthy that grip strength is influenced by many factors other than diet, which was not possible to control for in this study such as hereditary. However, the present results showed that dietary pattern analysis is a tool of a great value to examine the relation between diet and muscle function.

In interpreting the presented results it is important to note that handgrip strength may be affected by several factors. Age, body size, BMI, MUAC, energy intake, physical activity domicile and malnutrition risk have been controlled for in the present analyses. Obtained

results were similar to those of previous studies in terms of associations between handgrip strength and age, weight, height, body mass index and MUAC (392). There are also other variables for them it was impossible to control. Genetic is one of determinants of handgrip strength. Twin studies suggested a heritability of about 36% for handgrip strength in older adults (47;386;387). However, handgrip strength as a functional measure may be affected by some other factors, when measured. Position of the shoulder and elbow, room temperature, sensory deprivation problems, painful situations such as tennis elbow, psychological motivators such as colour of the room and music prior to the test may affect the results (478-480). The effects of these factors may be considered as random errors, which could lead towards a null result.

To my knowledge, this is the first study, in which associations between dietary patterns and handgrip strength were investigated with controlling for a number of possible confounders. Comparison to other studies therefore, is not possible in this respect. However, bivariate analysis presented in this chapter showed a non-significant association between dietary vitamin D and handgrip strength in men and a negative association in women (Table). This seems to be against previous studies indicating positive association between muscle function and vitamin D situation in both sexes. These results may be explained by the confounding effect of hand osteoarthritis (OA). OA is a common age-related disorder that is present in more than 10% of persons older than 65 years of age (473). Both handgrip strength and OA have been shown in most (399-401;474), though not all studies (406;407;475), to be associated with vitamin D situation. However, weaker handgrip strength in individuals who suffer from hand OA due to painful joints (476) may lead to an underestimation of the association between vitamin D and handgrip strength. If this is the case, the effect of other dietary variables may also be underestimated. It has been shown that handgrip strength can be improved in arthritic individuals by using non-steroidal-anti-inflammatory-drugs (NSAID) (477), implying that lower handgrip strength in such persons is due to pain and inflammation rather than muscle weakness. However, further analysis of the NDNS data showed strong correlations between handgrip strength and plasma vitamin D [25(OH) VitD] in men and women (Spearman correlation coefficients between 25(OH) VitD and grip strength were 0.30, P= 0.000 in men and r_s = 0.31,

P= 0.000 in women). These results were consistent with previous studies that reported positive association between plasma vitamin D and grip strength. Mowe et al (399) in a study of 349 men and women found a positive association between plasma vitamin D [25(OH) VitD] and grip strength (r =0.22, in hospitalised and r = 0.37, in home-dwellings, *P*<0.001). Similarly, Bischoff et al (400) found a strong association between plasma vitamin D and LEP (Leg extension power) in elderly men and women aged over 65 years.

Our results may be considered to be consistent with a randomised double-blinded supplementation trial that showed no benefit of oral administration of vitamin D in improving muscle strength in elderly men and women (407). In this study, 98 men and women aged over 69 years were supplemented with 0.5 microgram of $1,25(OH)_2$Vit D or identical placebo for six months. There was no difference between treatment and placebo groups after six months of supplementation, implying no association between vitamin D intake and muscle strength.

Given the strong association between plasma 25(OH)VitD and handgrip strength, lack of association or inverse association between dietary vitamin D and handgrip strength may be because of low contribution of dietary intake of vitamin D in plasma vitamin D in the study population. Dietary vitamin D intake in participants of NDNS was lower than RNI (10μg/d) in 97% of free-living people and 99% of individuals from institutions (mean intake was 3.4 μg in the total sample). Meanwhile, only six percent of men and 10% of women in free-living sample had plasma 25(OH)VitD less than 25nmol/l, a level that is considered as vitamin D depletion. In institution sample 38% and 37% of men and women were vitamin D-deplete. Dietary vitamin D had a borderline correlation with plasma 25(OH)VitD (r = 0.09, *P* = 0.02 in women and r = 0.21, *P* = 0.00 in men). However, positive association between healthy dietary pattern and grip strength in both sexes in the present study may suggest that the association between nutrition and muscle strength is beyond the effect of isolated nutrients.

8. Discussion

This thesis is concerned with the association between healthy diet with bone health and muscle performance in the elderly in the UK. These two issues are important factors influencing the risk of osteoporotic fractures. This thesis represents a secondary analysis of the data of National Diet and Nutrition Survey for people aged 65 years and over (NDNS) conducted on a UK nationally representative sample. The association between consumption of the healthy dietary pattern with ALP and muscle function in the elderly has been addressed in this study.

This chapter includes a discussion of the overall results in the context of the current literature, limitations and strengths of the study, possible explanations, the implications of the results for the public health nutrition and possible future work.

8.1. Hypotheses

The study hypotheses are restated below:

Primary hypothesis: Older people who eat a diet that complies with the healthy dietary guidelines have lower ALP and stronger handgrip strength than those eating a less healthy diet.

Secondary hypothesis: The effect of healthy diet on bone health is greater in those who had been optimally nourished in early life.

Tertiary hypothesis: The relationships between current diet, early nutrition with bone health and handgrip strength will be stronger in men than women.

8.2. Aim

To determine whether a diet that complies with the healthy eating guidelines is associated with lower ALP and stronger grip strength in the elderly.

8.3. Objectives:

In order to address the study hypotheses following objectives were considered:

For the primary hypothesis, objectives were:

1. To characterise eating behaviours and their determinants among the elderly population in the UK.

2. To explore the relationship between dietary patterns and plasma ALP as a marker of bone health.
3. To determine the relationship between dietary patterns and handgrip strength as a marker of muscle performance in older people.

For the secondary hypothesis, the objective was:

- To determine the effect of body size on the relationships between dietary patterns, ALP and handgrip strength.

For the tertiary hypothesis, the objective was:

- To identify the gender variations in nutritional aspects of bone health and muscle performance.

8.4. Assumptions of the study

- Plasma ALP is a marker of bone health and its increased level indicate the osteoporotic condition.
- Handgrip strength is a marker of muscle performance and its reduced level can lead to a fall.
- Body size, measured by weight and height, is a marker of early nutrition.

8.5. Methodology

Using dietary data of NDNS, seven statistically different dietary patterns were generated by principal component analysis and were characterised by their correlations with various food groups. Based on these seven dietary patterns, each subject has been attributed a dietary score on each of the dietary patterns and these scores were used in further analyses. Associations of dietary scores with nutrient intakes and other characteristics of the population were examined using correlation coefficients and analysis of variance, in men and women, separately.

Using multiple regression analysis, relationships between defined dietary patterns and plasma ALP were examined, while the effects of other bone affecting variables were controlled for.

Showing the usefulness of the eating pattern concept as a model for associating the dietary intake of various food groups to bone health, differences between discrete groups of body size within the elderly population with respect to the effects of diet on bone health were examined. Subjects were cross-classified by their thirds of height and weight in each gender. Stratified analysis was performed to examine the effect of body size on the relationships between diet and plasma ALP.

Using correlation coefficients and multiple regression analysis, association between handgrip strength and dietary patterns were examined. All analyses were performed for men and women, separately.

8.6. Key findings

The study achieved its objective of characterising diets of elderly people in the UK based on the dietary data of a national survey and showing relationships between these dietary patterns with ALP and handgrip strength. Seven dietary patterns extracted in this analysis accounted for 27.4% of variance of food use among the elderly population.

Multiple regression analysis identified the healthy diet, characterised by a high intake of vegetables, fruits, cereals, fish and other seafood, as the strongest predictor for serum ALP and handgrip strength in elderly men and women after controlling for energy intake, other dietary patterns and a number of confounding factors. Some eating patterns stood out of being significantly better or worse in relation to bone health or handgrip strength. It seems that the effect of healthy diet could not be explained by its nutrient contents.

Binary logistic regression analysis, controlled for energy intake, other dietary patterns and known confounders showed that from the lowest to the highest fourths of healthy diet intake, the prevalence odds ratios of high ALP, defined by median, decreased by more than half in women (OR = 0.43, 95% CI, 0.3-0.7) and by two thirds in men (OR = 0.35, 95% CI, 0.2-0.6) with a statistically significant trend in both sexes (*P* for trend <0.001).

Classifying individuals by their highest dietary scores showed that only 11% of men and 18% of women are frequently practicing the healthy diet. In institutions only 1% of men and 2% in women can be classified as "healthy eaters".

Using similar model of multiple regression analysis across the groups of weight and height revealed different associations between dietary patterns and ALP in men and women as below:

- Among men the strongest positive association between "healthy diet" and bone health was seen in the heavy-tall (HT) group (B = -0.04, $R^2 = 0.18$, $F_{(1, 84)} = 17.5$, $P<0.001$). For those within the short and thin group, the association between healthy diet and ALP did not reach to a statistically significant level.
- Among women, healthy diet was associated with ALP in the entire sample (B = -0.02, $\beta = -0.14$, $R^2 = 0.023$, $P = 0.00$), but when the sample was stratified by height and weight the association between healthy diet and ALP failed to reach to the statistically significant level among all weight and height groups. Instead, various dietary patterns found to be important in predicting ALP in different weight and height groups. For example; in females within in the first third of height and weight (thin-short group), the sugary-dairy diet, which was characterized by high intakes of dairy products, canned fruits and sweet foods, was more related to ALP than other dietary patterns in a protective way. In females who were tall and heavy, on the other hand, the vegetarian-trend diet, characterized by high intakes of not fried potato products, leafy green vegetables, carrots, peas and green beans was associated with ALP in a detrimental way (B = 0.04, $\beta = 0.30$, $R^2 = 0.07$, $P = 0.01$).

8.7. Relation to other studies

The approach of principal component analysis was used previously in several studies to identify dietary patterns. Dietary patterns derived in this study were qualitatively similar to those of other studies. Randall et al (410;428) used the data on consumption of 110 foods from which nine dietary patterns were derived and relationships between identified dietary patterns and cancer risk were examined. The "healthful" pattern in their study was related to the consumption of green leafy vegetables and low-fat animal products. In another study using the data of the dietary and nutritional survey of British adults, Gregory and collaborates (418) identified five dietary patterns that were closely similar to those of mine. The healthy eating pattern in their study was strongly associated with the consumption of vegetables, fresh fruits whole meal breads, reduced fat milk and low fat spreads.

Our study also confirmed the results of other studies showing that dietary information can be summarised to a limited number of eating patterns, reflecting healthful and less healthful dietary patterns (412;416;429-431).

Since this is the first study on relationships between a marker of bone health and dietary patterns, comparison with other studies in this regard is not possible. However, our findings are somewhat consistent with associations between nutrient intakes and bone health identified in previous epidemiologic studies in particular, calcium, protein, vitamin D and C, phosphate, copper, zinc and potassium. Frequently, previous studies were focused on isolated nutrients and the effects of nutrients were assessed in men and women combined. Furthermore, more specific criteria of bone health were used, such as BMD, BMC or fracture risk and these criteria are affected by variables differently, according to the site of skeleton, on which the effects were assessed. Therefore, comparison to other studies sometimes may not be possible or informative.

Weak relationships between dietary variables and bone health among women, observed in our study, is consistent with the findings of others in previous studies, especially when a study was conducted in men and women separately or when postmenopausal women were compared to pre-menopausal ones (105;155;159). Some studies in this regard, however, showed positive relationships between bone health and intakes of some nutrients among women (106), which are not consistent with our results. Munger et al (106) in a 3-year prospective study found that the risk of hip fracture in postmenopausal women, of the age of 55-69 years, was negatively associated with protein intake but was not related to the intakes of vitamin D and calcium. However, differences in methodologies and populations made it difficult to compare the results. Their study was prospective, and the outcome measure was fracture risk and the population was younger than our population. Lack of association of calcium and vitamin D with fracture risk was consistent with our results.

Among men the positive associations between protein, calcium, vitamin D and bone health are consistent with most previous studies (155;159) but not one of them (160).

To my knowledge, this is the first study in which the relationship between muscle function and dietary pattern has been addressed, therefore, comparison to other studies is im-

possible. However, some of my results are comparable with other studies, for example, strong correlation between handgrip strength and muscle mass, measured by mid-upper arm circumference, and body size measures (height and weight), observed in this study are consistent with other studies mentioned in Chapter two (392-394). In addition, negative association between age and handgrip strength, observed in this study, was reported by a number of studies (377-380).

8.8. Possible explanations

8.8.1. Dietary patterns

The findings of this study indicate that dietary patterns may be more related to bone health and handgrip strength than any isolated nutrients or foods by its own. It may be explained by the fact that diet is a complex combination of a great number of nutrients and non-nutrient components. These components are consumed together and may interact with each other. Interactions during absorption, transportation and utilization by tissues are sometimes too complex to be considered for each nutrient, separately, especially when their contribution to a multifactorial condition such as bone metabolism is of interest. The best example of such interactions is the effect of vitamin D on calcium and phosphate metabolism. Vitamin D is a major determinant for effective intestinal absorption of calcium and phosphate (79), for reabsorption of calcium in the distal convoluted tubules and excretion of phosphate in the urine (17) and for mobilisation and deposition of calcium in the bone (17;79;81). Conversely, plasma phosphate and calcium can affect renal production of $1,25(OH)_2$VitD from its precursors coming from diet or skin (64). Calcium balance and its bioavailability is also influenced by nutrient and food interactions; in this respect, vitamin D and C, lactose and glucose are major favouring factors and polyphenols (tannins in the tea), excess of phosphorous, iron, and zinc in proportion to calcium, phytic, oxalic and fatty acids as well as dietary fibre are limiting factors for calcium absorption(5). Caffeine can increase urinary excretion of calcium and induce a negative calcium balance (209;210). With respect to bone metabolism, many nutrients play a role in relation to health with none of them being effective in isolation, and their effects on bone health are likely more than can be accounted for by any single nutrient. Therefore, it may be invalid to consider single foods or nutrients in relation to bone health without consider-

ing the complex interaction and correlation of all nutritional and non-nutritional components of the diet. In this regard, pattern analytic approach seems to be more comprehensive for considering these interactions, which are frequently unknown. Additionally, because of the integrity of body metabolism, optimal performance depends on the presence of all necessitous dietary factors. Therefore, stronger correlation between dietary pattern and bone health is not surprising. In this respect, the effect of healthy diet may be explained by its food contents and the way that variety of foods are consumed.

8.8.2. The effect of body size

The results of the present study suggest that the relationships between diet and bone health may be modified body size. Associations between dietary patterns and ALP differed in people with different body weight and height, with stronger positive association between healthy diet and bone health in taller and heavier persons, especially in men. The underlying biology of our findings on body size is unknown but there may be several possible explanations. Noteworthy, body size may operate through several, not mutually exclusive, mechanisms.

Both bone mass and height may have pre and postnatal determinants (42;267;481;482). To some extent, these early determinants may act through metabolic procedures. Although most of such suspected procedures are yet unknown for us, there are examples, which clearly implicate early determination of some metabolic procedures, such as programming of several hepatic enzymes of fuel metabolism (253) and programming of the hypothalamic-pituitary-adrenal axis (264;265). However, permanent alteration in endocrine profiles may at least in part underlie the observed associations. Persistent low levels of IGF-I and some other hormones such as insulin and TSH (thyroid stimulating hormone) together with increasing levels of GH in growth retarded children suggest the early programming of endocrine profiles and metabolic procedures (263;485;486). Insulin is important for glucose utilizing and protein synthesis (17;251). Lower insulin production and lower insulin response (i.e. raising of plasma insulin activity following glucose injection), is associated with lower growth in childhood and shorter stature, thereafter (266). Insulin is also positively associated with bone mass (483). TSH is a major determinant of fuel and protein metabolism (51) and is important for skeletal homeostasis and linear

skeletal growth (17;51). IGF-I is promotes protein synthesis and inhibit bone collagen degradation in bone (17). It is also a major regulator of vitamin D metabolism and intestinal calcium absorption (56;57). Estradiol and testosterone can increase the plasma level of $1,25(OH)_2$VitD via increasing 1-α-hydroxylase and suppression of 24-hydroxylase in the kidney (17). Protein synthesis, fuel metabolism, calcium absorption and vitamin D metabolism and their actions on bone are examples of metabolic procedures that can be altered by early exposures, and their early determinants may be reflected by body size. On the other hand, endocrine profiles can be influenced by nutritional exposures. For example; protein and vitamin D promotes IGF-I production (55). IGF-I is also associated with variety of food intakes including red meat, carbohydrate and fat (484).Also, enough protein intake is necessary for the action of sex hormone and PTH on bone (91). These interactions between programmable endocrine profiles and dietary intake suggest the complex interactions between early and late nutritional exposures regarding to bone health. Infants with better early nutritional experience, reflected by their body size, may be more efficient with respect to their endocrine and metabolic profiles. However, a recent published study reported an inverse association between birth weight with calcium absorption and plasma vitamin D (188). Among 322 postmenopausal women participated in a twin study, women with highest quintile of recalled birth weight had 16% lower fractional calcium absorption than those within the lowest quintile of birth weight. Adjustment for a number of variables including; age, serum concentration of calcium, 25(OH)VitD, creatinine, phosphate and PTH did not change these associations but when the plasma level of vitamin D ($1,25(OH)_2$VitD) was included in the model, the association became non-significant, indicting that the association between birth weight and calcium absorption is partly due to the effect of vitamin D. It seems that lower birth weight leads to up-regulating calcium absorption via affecting renal production of vitamin D. It may also be due to gene-environment interactions in early life.

Body size serves partly as an indicator of socio-economic circumstances and nutritional status in childhood (40;41;267-269;274), with taller people more likely to have been nourished optimally during their growth period than shorter ones (459), and this may partially underlie the observed effects of body size on the associations between dietary patterns and

bone health. Taller persons are more likely to have had better bone gain during their peak bone mass because of better nutrition and longer exposure to sex hormones due to earlier spurt and earlier menarche in females (156;270). Higher growth in childhood and bigger body size may also indicate higher stimulation from growth factors such as IGF-I (458), which are important regulators of both somatic growth during childhood and bone metabolism in later life (52;54;91). However, how these exposures during peak bone mass may be translated to modification of dietary intakes in older ages is remained to be determined.

The effect of body size on the relations between dietary patterns and bone health may be partially explained by variables that may differ between the groups. As Table 7 Appendix 2 presents heavy-tall persons were more likely to be from social class I and II than thin-short persons (40% vs. 27%) and were less likely to be from social class IV and V (17% vs. 24%). This difference between groups may in some part, explain the obtained results. Although presented analyses were controlled for social class differences, it may not remove the effect of social class, completely. It is well documented that people within lower social class have poorer health status and may suffer from various illness more than people from higher social class (487;489). Poor health condition and using medications may affect their lifestyle, eating behaviour and the relations between diet and health. Moreover, the risk of mortality in people from lower social class is about three times of that of others within the higher social class (490;491), which can in turn, lead to shorter life expectancy in those from lower social class (492-494). Earlier death in this group may lead to a selective survival into old age of those who are healthier and are better nourished and therefore over estimated the effect of healthy diet in heavy-tall persons. However, for more lifestyle variables (e.g. illness, smoking, activity), which may vary by weight, height and social class, analyses were controlled for, and therefore it is unlikely that the total associations seen in the study be due to these differences.

Adult body size, as stated earlier, is determined primarily by two factors: heredity (heritability values of 0.75-0.78 (455)) and nutritional exposures experienced during development. It is possible that hereditary factors resulting in a certain body size may lead to the observed effect of body size on the relations between eating patterns and bone health. For

the environmental factors, which are likely to be associated with bone health and were available in this study (e.g. lifestyle and demographic variables and bone-affecting nutrients such as vitamins and minerals), and may differed between w/h groups, all analyses were controlled for, and therefore, could not readily explain the observed effects of weight and height. To some extent, the differences between groups may be explained by genetic factors. Bone mineral status in all stages of the life is under major influence of genetic factors, from childhood to the elderly. Estimated heritability of bone mineral density is about 0.46 to 0.84, depending on the skeletal site measured (286) and different genotypes have been described in this regard. The vitamin D receptor (VDR) genotype is supposed to be one of the major determinants of bone mass (75), although inconsistent results have been published. VDR is a major regulator for the calcium homoeostasis in the body. A twin study in Britain showed a strong influence of the vitamin D receptor genotype on bone density at various skeletal sites, including the hip, lumbar spine, forearm and the whole body, after controlling for age, weight, years since menopause, and use of HRT (75). Similarly, a study of postmenopausal women reported significant associations between VDR genotype and spinal BMD in healthy and osteoporotic persons (76). However, others reported no association between VDR genotype and bone mineral measurements (78;150;151). Laskey et al (151) found no relation between VDR genotype and changes BMD of the whole body, spine, hip, and forearm among breast-feeding mothers. Arden et al (78) also found no relations between calcaneal bone measurement and VDR polymorphism in 189 pairs of healthy dizygous twin females.

However, there is evidence that the relations between VDR genotype and bone health is subject to modification by current and early nutritional exposures. Keen et al (150) found significant association between VDR genotype and weight at one year (7% higher 1-y weight in homozygote "tt" in comparison with homozygote "TT") ($P = 0.04$) in 66 elderly females, suggesting interaction between genetic and early environmental exposures on infant skeletal growth, which may track through adult life. A recent investigation on 291 men and women aged 60–75 years showed that intrauterine under nutrition may modify VDR influences on bone (73). In this study, associations between VDR genotypes and BMD at lumbar spine and proximal femur varied according to birth weight. Among sub-

jects in the lowest third of birth weight, those with the "BB" genotype had higher spinal BMD ($P = 0.01$), while among those in the highest birth weight "bb" genotype accompanied by a higher spinal BMD ($P = 0.04$). Similar associations were found between spinal BMC and VDR genotypes in low-birth weight group. Associations were adjusted for age, sex and weight at baseline.

Allied to the effect of early nutrition, current diet may also modify the effect of VDR genotype on bone (148;149). Graafmans et al (149) reported more beneficial effects of vitamin D supplementation (400 IU daily for at least 2 years) on femoral BMD among subject with "BB" genotype compared with those with the "bb" genotype ($P = 0.03$) in elderly women. Similarly, it was found that VDR genotype associated with bone loss at femur only in those with low calcium intake (mean = 376 mg/day (148)). These findings suggest that both body size and VDR function or the expression of VDR gene are influenced by early environmental exposure and may affect the relations between diet and bone health. Therefore, effects of weight and height on the relations between diet and bone health may be partly because of different expressions of genetical factors due to early environmental modifiers.

Another possible explanation for the findings of the modification effects of body size on the relationships between diet and bone health may be the effect of body composition. Height is related strongly to lean body mass (439) and weight is related to BMI and fatness (440). Among men, after adjusting for differences in body mass, leanness (a higher lean to fat ratio) is associated with higher bone mineral status at several skeletal sites (495). In postmenopausal women, on the other hand, fatness (a lower lean to fat ratio) is positively associated with bone mineral status (496). Fat mass may be determinative for endogenous production of oestrogens by adipose tissue, which may be particularly important in women after the menopause (441). And lean body mass may be related to the osteogenic effects of muscle on the skeleton. Both fat and lean body mass are important in shock-absorption and diminishing the energy of a fall.

The modifying effect of the body size on the relationships between diet and ALP may also be explained by variation of nutritional demands and the role of limiting nutrients. As we observed in this study, individuals with different body sizes have different eating behav-

iours and these differences may lead to different nutritional states and dietary demands. However, because dietary patterns can not be specific about the particular nutrients, it is impossible to address this issue by pattern analytic approach.

However, there is still much to be learnt about the way in which body size may affect the relations between diet and bone health in the elderly. What the results implicated is that the relation between diet and bone health in the elderly vary by body size and therefore people with different body size should be advised differently.

8.8.3. Gender effect

The results of the study indicate that in general, dietary variables were less associated with ALP and grip strength in women compared to that of men.

Sex differences in the relationships between diet and bone health may be because of different nature of osteoporosis in sexes. As discussed in the first and second chapters, there are two distinct syndromes of osteoporosis; Type I and II. . Type I osteoporosis is referred to as the rapid bone loss and is usually common in postmenopausal women, among them bone is affected by huge deterioration of sexual hormones, which can lead to an increase in bone turn over. In this syndrome, it seems that in the causality pathway, the priority may be with hormonal changes rather than nutritional variations. Type II osteoporosis, on the other hand, is associated with a slow phase of bone turn over and bone loss. This syndrome has been attributed to age-related factors, which may affect bone metabolism by impairing calcium homeostasis and vitamin D metabolism and possibly gradual deterioration of oestrogen and testosterone (506). Both types can be seen in females whilst men do not have the rapid bone loss syndrome. Our results may be considered to agree with previous studies on these two syndromes (507). Rico et al (507) in their study on 40 women with type I osteoporosis and 20 women with type II osteoporosis, reported significantly lower concentrations of three nutritional markers (i.e. transferin, retinol-binding protein and prealbomin in type II osteoporosis than type I osteoporosis, although both groups had lower levels of these three markers in comparison to two normal groups, who were age matched with osteoporotic subjects. Therefore, weaker associations between dietary patterns and bone health in women in this study may be attributable to higher frequency of type I osteoporosis in women, which may last long time after menopause (173).

The results showed that the effect of body size on the relations between dietary patterns and ALP varied by the gender. For example, among men with greater body size, the healthy diet was strongly associated with ALP but this correlation did not reach to a statistically significant level in corresponding group of women. To explain why heavy-tall women did not benefit from healthy diet, it is possible that the linear growth in women be less affected by early nutrition in comparison to men, and therefore, early nutrition is not properly represented by body size in women (274;446). Therefore, one of the assumptions of the study that body size is a marker of early nutrition may not be true in women. Additionally, as stated above, bone metabolism in women is mainly affected by sex hormones. It is possible that the critical period for programming of the body size and sex hormone profiles be different in women, therefore, determinants of body size at the time of programming may be different from those of sex hormone programming.

In this respect, it is noteworthy that the relations between dietary variables (in terms of either dietary pattern or nutrient intake) and ALP in women were weak. Stratifying the sample reduced the sample size and attenuated these associations; therefore, they could not reach to a statistically significant level only because of power issues.

However in interpreting the results it should be brought to mind that correlation does not imply causation. Although the associations of certain dietary patterns with ALP and handgrip strength seemed to be strong, some care is needed to conclude that these associations are cause and effect relationships. Such interpretation of cross-sectional studies is impossible.

It is also important to note that dietary patterns generated in this analysis accounted only for about 27% of variation in food intakes among the sample, indicating the possible existence of further patterns or dietary factors. However, this proportion of variance in food intake is somewhat similar to the results of other studies (418).

The last important point is that bone metabolism is influenced by many factors other than nutritional ones and for some people the causal priority may be with factors other than nutritional factors. Therefore, the fact that dietary patterns may explain a small proportion of ALP variability is not surprising.

8.9. Advantages and disadvantages of principal component analysis.

8.9.1. Advantages

Main advantages of principal component analysis are as below:

1. In this approach, the effects of all nutrients and foods as well as other components in a usual diet would be taken into account.
2. With respect to the integrity of body metabolism, pattern analysis seems to be more plausible than single-nutrient analyses.
3. Using pattern analysis, it would be much easier to understand the food preference among the population with regard to other lifestyle and demographic factors. This may have implications for improving the eating behaviours in order to establish a healthy life in the elderly.
4. This approach may be of benefit for nutritional education and for setting the public health nutritional strategies.
5. The results of this approach are much easier to be translated to the public and making nutritional advice. It is also parallel to the real world, in which people eat foods not nutrients.
6. Using the pattern analytic approach in nutritional research, particularly about chronic diseases of multifactorial aetiology, may produce more reliable results

8.9.2. Disadvantages

Disadvantages of the approach of principal component analysis are as below:

1. Dietary patterns are likely to vary from one population to another. Thus, the results of factor analytic approach in a population may not be applicable to other populations.
2. Pattern analysis is data dependent and food groups used in the analysis play a major role for identified dietary patterns. Using different grouping scheme may be resulted in different dietary patterns among the same population.
3. The biological bases for the relations between dietary patterns and bone health is difficult to be addressed, because dietary patterns can not be specific about the particular nutrients responsible for the observed associations. In this study, most nutrients were broadly related to the first three dietary patterns, less or more, but the strongest predictor of bone health was the first dietary pattern in both sexes. Among women, none of

nutrients were related to bone health but the first dietary pattern was still a predictor of bone health.

8.10. Study Limitations

There are a number of limitations to the study that could affect the results and subsequent conclusions. These limitations will be discussed with respect to their possible effects on the results.

8.10.1. Chance

In assessing the findings of the study it is important to know whether the results obtained could have occurred by chance alone. The level of significance, represented by *P* values, will determine how likely it is that the findings occurred by chance if there were no real association. This is the function of sample size and the variance of the estimate. The smaller the variance or the bigger the sample size, the more likely is that the observed association be significant and the less likely is to occur by chance (94). Conventionally, if *P* value is 0.05 or less, it will be concluded that the results could not be occurred by chance. The results of this study were considered significant if the *P* value was smaller than 0.05. Although such results are considered to be significant, which can not be obtained only by chance, it is still possible to happen by one out of twenty by chance. However, the results of multiple regression analyses for both ALP and handgrip strength were significant with *P* values of between 0.00 and 0.02, with most of them being 0.00, suggesting that the obtained results were very unlikely to be obtained solely by chance. More importantly, the results are biologically plausible as discussed in above sections. Furthermore, the findings are consistent with other studies linking dietary pattern to the occurrence of chronic diseases (409-412;416).

A further issue in this regard is the power of the study, which is the probability that a non-significant result be actually true. According to the type of the analysis that will be undertaken, different equations can be used to calculate the power of the study (94).

To calculate the power of the study for association between continuous variables (dietary scores with ALP and handgrip strength) the following formula can be used (94):

$$Z_{1-\beta} = \sqrt{\frac{d^{*2}(n-5)}{1-d^{*2}}} - Z_{1-\alpha/2} \qquad \textbf{Equn.8. 1}$$

Where $Z_{1-\beta}$ is the power of the study, $Z_{1-\alpha/2}$ is the significance level, n is the sample size and d^* is the correlation coefficient, which has been detected.

Using this formula, as expected, for the statistically significant correlations, the power of the study was high enough to detect the claimed associations. For example, the power of the study to detect the correlation coefficient of -0.17 between healthy diet score and ALP in the entire sample (see Table 5.1), where n = 1107, d^*= -0.17 and P = 0.00($Z_{1-\alpha/2}$ = 2.58, known from (94)) was calculated to be more than 95%. For non-significant associations, on the other hand, it is important to decide which sample size is needed to detect a claimed correlation at a statistically significant level (say P value of 0.05). To estimate sample size needed for these coefficients to be significant, following formula can be used (94):

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2}{d^{*2}/(1-d^{*2})} + 5 \qquad \textbf{Equn.8. 2}$$

Where $(Z_{1-\alpha/2} + Z_{1-\beta})^2$ for a significance level of 0.05 and the power of 80% is known from reference (94) to be 7.8.

Calculated minimum sample size needed to detect claimed correlation coefficients (Table 5.1) at a statistically significant level and with the power of 80%, was between 2,163 to 77,997 subjects. Rather similar figures were obtained for handgrip strength; to detect a correlation coefficient of -0.06 between grip strength and the sixth dietary pattern in women (see Table 7.2), at a statistically significant level would have required a sample of over 2,164 females. Similarly, among men to detect a correlation coefficient of 0.01 between grip strength and "vegetarian-trend diet" at a statistically significant level, at least a sample of 77,993 men would be needed. Similar figures were obtained for the results of multiple regression analyses. These figures show that the real associations between dietary patterns with both handgrip strength and ALP for those patterns with non-significant correlations were actually very weak. Such associations may not be biologically plausible. On the other hand, for patterns, which made a significant contribution to the variance of

both ALP and handgrip strength, the power of the study was high enough to detect the real effect.

8.10.2. Bias

When considering the findings, it is important to assess the effect of errors. Errors may lead to a wrong answer and can be divided into two general classes: random errors and systematic (or differential) errors. Random errors will generally affect the entire sample in a no predictable direction and therefore will lead towards a null result. Differential errors, on the other hand, will affect the sample in a direction by which the results of the study could be over or underestimated. Differential errors are referred to as "bias". Errors can be a function of sampling methods, reporting dietary intakes by individuals, coding of dietary data using food composition database, analysing the data or interpreting the results.

Selection bias may be present in the survey (NDNS) if respondents were different from non-respondents. As stated in section 3.2.4, subjects, who fully co-operated with the study, were different from others, who failed to fully co-operate, in terms of sex and age profiles and marital status. In both free-living sample and the institution sample, respondents compared to non-respondents were more likely to be male, younger (by an average of 1.2 years), married and not living alone. They were also more likely to be from social class I and II. Differences between respondents and non-respondents may have affected the results in the way that younger individuals from higher social class may have some idea about healthy and unhealthy diet and therefore, reported a more desirable diet. This is referred to as "social desirability bias"(94). However, using dietary records may have reduced the possibility of this sort of bias, although recording of diet by participant, by itself, may have influenced eating behaviours, and lead to a report that vary from usual diet. Therefore, it is possible that the proportion of healthy eaters be overestimated and observed associations be exaggerated. I have no way to confirm or deny this possibility.

Using proxy information may introduce a further bias (information bias). Information of 34% of people from institutions and 3% of free-living people have been provided by the proxy information givers and this may lead to misreporting the dietary intakes and other information related, for example; to the assessment of physical activity, malnutrition risk, health history and smoking history. In institutions, mostly carers were the proxy informa-

tion givers and this may lead to a tendency of reporting more healthy diet to show better cares in the selected institutions. Such misreporting may lead to an overestimation of the prevalence of healthy diet in institution people. However, comparing the proxy information with those reported by the respondents in institution sample showed lower percents of healthy eaters in those whose information were provided by proxy compared to those whose information were reported by themselves (2.7% vs. 1.2). Therefore, overestimation of healthy diet by proxies is very unlikely. Since the proxy was used when a person was deemed, from the results of memory questionnaire, or was unable to provide the information required, and such cases were more likely to be ill (90% vs. 10%) lower healthy diet in proxy information givers than respondent information givers was not surprising, and under-reporting of healthy diet in proxy information givers also seems to be unlikely. However, excluding proxy information givers in both free and institutional sample did not change the results of multiple regression analysis for both ALP and grip strength, substantially. In institutional sample (men and women together or separate) the model of multiple regression analysis for ALP [the same model used before (see section 5.3.1)] was still non-significant and for grip strength showed rather similar results for men [$\Delta R^2 = 0.17$ df = 27, $P = 0.02$ (compare with Table 7.3)] and was still non-significant in women. Because of power issue and sample size, it was impossible to do similar analyses in proxy information givers. In conclusion, it is very unlikely that dietary misreporting by proxy information givers in both free-living and institution sample biased the obtained results.

It is very unlikely that biased information being reported by interviewers or participants due to awareness of their bone health situation or the plasma ALP, because survey was not primarily targeted to assess bone health or osteoporosis. However, errors of coding the questionnaire by interviewers can be considered as random errors.

A further bias may be introduced by misreporting the dietary intakes by heavy people. They may tend to report lower dietary intakes and more healthy diet and this may lead to an overestimation of the effect of healthy diet. If this is true, it would be expected that heavy-short people be as concerned as were the heavy-tall people and therefore they may also tend to report a more healthy diet. Comparison between these two groups showed

that this information bias was very unlikely, because only 3.6% of heavy-short people were healthy eaters whilst in heavy-tall group 20.4% of subjects were classified as healthy eaters.

Selective survival into old age of those who are at lower death risk by any reason, may lead to a biased results. If osteoporotic people were at higher risk of death (e.g. due to complications of osteoporosis or poor health conditions) and non-osteoporotic subjects be at lower risk of death, it may have affected the results. Presented analyses indicated that people with healthier bone had higher intakes from healthy diet and those of poorer bone health had lower intakes from healthy foods and higher intakes from sugar, puddings and unhealthy foods. If severe osteoporotic people, who have already died, were healthy eaters, with high scores on healthy diet, then the effect of healthy diet may be exaggerated, because the number of those with healthy diet and severe osteoporosis are underestimated. Conversely, if severe osteoporotic persons had higher intakes from unhealthy foods and lower intakes from healthy foods and have already died the effect of healthy diet may be underestimated. However, because this is a cross-sectional study, it is impossible to say exactly how the results could have been affected by the survival bias. However, socio-economic class was shown in many investigations to be related to the risk of death (490;491) and all analyses were controlled for the social class and therefore, it is unlikely that this type of bias have affected the results, substantially.

The last point that may lead to a bias is that if a person with diagnosed osteoporosis has changed his diet, recently. In this case, because of the time lag between the diet and its effect on the development of a chronic condition such as osteoporosis, current diet may have limited relationship with present disease. Therefore, if the diet is just recently changed to a healthy diet, the effect of previous unhealthy diet may be considered as the effect of healthy diet. This can underestimate the effect of healthy diet because proportion of osteoporotic persons with healthy diet will be overestimated. Because of lack of date, I have no way to say how much the results may be affected in this way.

8.10.3. Other confounding variables

Although a number of confounders were taken into account for the analyses, some others have not been accounted for. For example no information about menopause, puberty and

peak bone mass were available in the data used throughout this thesis. It is well documented that bone health in the elderly is determined by the amount of bone mass gained during growth (peak bone mass) and the rate of bone loss by the age. In this study, however, data of exposures during growth were not available. Genetic is also a major determinant for both bone mass and muscle function. No information was available in this regard. In addition, since 99% of the sample population was selected from white ethnic group, the effect of race and ethnicity on both exposure and outcome could not be addressed.

8.10.4. Other considerations

For interpretation of the results, it is important to note the following considerations:

- Because it was a cross-sectional study, drawing causal inferences from the data is limited and therefore obtained associations should be interpreted with caution.

- Plasma alkaline phosphatase was used in this study as a marker of bone health. When considering the findings, it is important to note that total ALP is not originated exclusively from bone tissues. It can also come from other sources such as biliary system in liver. Increasing levels of alkaline phosphatase might be seen in primary or secondary (metastatic) bone cancers and biliary obstructions. Therefore, ALP is not specific to bone metabolism. However, ALP is a well-established marker of bone turnover, which is commonly used in clinical and epidemiological studies of bone metabolism and has been found to be strongly correlated with the bone-specific ALP (B ALP) in both healthy and osteoporotic individuals (318,327,339,340,342). Furthermore, several studies have noted that both B ALP and T ALP are well correlated with bone mass measurements in older people (173;319;320;331;333;335) as well as bone turnover, which is a major determinant of bone loss in the elderly (173;320;344). There is evidence indicating that in the absence of liver disease, there maybe no preference of B ALP over T ALP in the osteoporotic patients (339,345). Therefore, ALP can be of appreciable value to assess bone activity in epidemiologic studies of osteoporosis (313).

- A further issue in this regard is that in relation to osteoporosis, ALP is a general and a crude marker of bone health throughout the skeleton. Because it is not specific to any of skeletal sites, high levels of ALP will not necessarily indicate the presence of osteoporosis in any skeletal site. However, as stated above, increasing ALP indicate a

high level of bone turnover and high probability of osteoporosis or poor bone health. In this respect, the finding of the study regarding to the associations between dietary patterns and ALP have no implication for the effect of diet on specific skeletal sites.

- Another important consideration in interpretation of the results is that the patterns identified in this study are the present dietary intake, which seems to have a limited relationship with present disease incidence, because of the time lag between the diet and its effect on the development of a disease, which may be considerable. Furthermore, osteoporosis is a chronic disease resulting from a long-term effect of different variables including nutritional and non-nutritional ones, which may have been subject to changes during the time.

- The last point to mention is that all dietary patterns identified in this analysis accounted for only about 27% of variation in food consumption, indicating the potential existing of other patterns or factors.

8.11. Implications of the study

The results suggest that eating patterns may be more related to bone health than any isolated nutrients or foods by its own and the effect of these patterns are influenced by body weight and height, two indicators of body size and long-term nutritional state. A healthy dietary pattern characterised by a high intake of vegetables, fruits, cereals, chicken, turkey fish and other seafood is beneficial for bone health and muscle function in older ages.

If the results of this study are valid, the traditional single-nutrient approach by which more emphasis would be placed on some foods or single nutrients may not lead to the results that would be expected. Efficiency of nutrient intakes through diet is necessary for health, but it does not mean that a nutrient-efficient diet is necessarily healthful and efficient to provide good health for the public. In this respect, it seems to be appropriate to move from nutrients to the patterns of food intake in setting nutritional policy. Thus, traditional approach of making policy, considering the efficiency of nutrients, may not be compatible with good health for the public. It is necessary to review current nutritional policies with more comprehensive methods.

It is important to note that the "healthy diet" identified in this study, was compatible with the guidelines of the healthy eating pattern in the UK (448). Consumption of variety of foods, having a plenty of fruits and vegetables in the diet, eating plenty of foods rich in starch and fibre, avoiding the consumption of too much sugary foods and drinks and not eating too much from foods that contain a lot of fat are the key points of the guidelines which are compatible with the characteristics of the "healthy diet" identified in the present study. The results indicate that a diet which fulfils the requirements of the current guidelines is compatible with good bone health and muscle function in the elderly in the UK.
By using logistic regression analysis, it was found that increasing healthy diet scores from the lowest quartile to the highest within the ordinary distribution of dietary intakes in the UK could decrease the risk of high ALP to the third in men and to less than half in women, adjusting for a number of confounders.

According to the results of this study, "healthy diet" would not be of benefit for some subgroups of the population. In general, none of eating patterns could be considered as healthy diet for all subgroups of the population and, therefore before going to the public, it is necessary to set strategies to target subgroups and decide which diet may be of benefit for any of the population subgroups. In this study, body size was selected to stratify population, because it was found that body size may act as an effect modifier, but it may also be possible to consider some other characteristics to target population groups, such as lifestyle variables or ethnicity.

Differences of the prevalence of healthy eating by various lifestyle variables, seen in this study can be used as an indicator for targeting population subgroups within the framework of public health nutrition policies. It was shown that smokers, for example; are more likely to have unhealthy diet, therefore, it may be a prior target to improve their diet.

Associations between life style variables and dietary patterns, seen in this study may indicate that prior to diet other factors may need to be changed such as smoking habits or activity levels. Therefore, lifestyle improvement may be also considered as nutritional policy in the public health nutrition context.

8.12. Future work

The findings of this study suggest that pattern analysis may be a useful tool for investigation the association between diet, ALP and muscle function in the elderly. However, because results of the present study are sample-specific, it may need to be confirmed by further investigations in different populations with more specific markers of bone health, such as fracture risk or bone mass measurements. Additionally, to be considered causal, associations between healthy diet and bone health should be observed consistently across a number of population-based studies, preferably by prospective investigations.

As stated before, relation between various factors with bone health and osteoporosis is site-specific, which could not be properly addressed by considering a general marker of bone metabolism such as ALP. Therefore, it may be recommended that the results be confirmed by future works by using more specific indicators of bone health, such as fracture risk or bone measurements for specific skeletal sites.

The results showed that diet could explain an appreciable proportion of the variance of ALP and handgrip strength, but not the total of their variance. In fact, genetic polymorphism, life style variables and hormonal status are also important predictors of bone health and muscle performance. Therefore, it is necessary to investigate the interactions between genetic polymorphism, lifestyle variables, hormonal status, dietary patterns and early nutrition in a larger population dataset.

If differences in associations between dietary patterns and bone health are related to the programming phenomenon in early life, it is important to find the most important determinants of bone metabolism programming.
In women, it is particularly important to find the major determinants of sex hormone programming in early life and to identify a proper marker for such determinants. Having a relevant marker may be of value for targeting women most at risk for osteoporotic fractures. Such marker may be used to determine which dietary pattern is of more benefit for each population sub groups.

For most analyses among subjects from institutions, the relations between dietary factors and ALP did not reach to a statistically significant level. It may be due to low variability

of the dietary practice in these people because of limited food choice in the institutions. Additionally, reported data from people living in institutions may not be enough precise, because a major part of information obtained from proxy information givers. Because of increasing concerns of the nutritional deficiency in institutions, it may be recommend conducting further research in this group to ensure that their nutritional demands are properly satisfied and appropriate nutritional policies have been set for them. The results of this study showed that a majority of these people have practiced the sugary-dairy diet, which is not consistent with the current dietary guidelines in the UK. However, because of low variability of dietary intakes the results may not be reliable in this group, calling for further research to determine; firstly which dietary pattern may be more beneficial (or healthy) for them, and, secondly how their demands is currently fulfilled.

8.13. Conclusion

This study has examined the effect of a diet, which broadly complies with the current guidelines in the UK for a healthy diet, on bone health and muscle function in the elderly. The main hypothesis of the study was "Older people who eat a diet that complies with the healthy dietary guidelines have lower ALP and stronger handgrip strength than those eating a less healthy diet". The second hypothesis was "The effect of healthy diet on bone health is greater in those who had been optimally nourished in early life". The third hypothesis was "The relationships between current diet, early nutrition with bone health and handgrip strength will be stronger in men than women". Findings of this study showed that after controlling for known confounders and various nutrients, the strongest predictor for ALP and handgrip strength in both sexes was a diet, which was consistent with healthy dietary guidelines in the UK. Therefore, based on the analyses presented in this thesis, the primary hypothesis should be accepted.

Assuming that body size is a marker of early nutrition, stratified analysis by height and weight showed that among men the most beneficial effect of "healthy diet" on bone health was seen in the heavy-tall (HT) group ($B = -0.04$, $R^2 = 0.18$, $F_{(1, 84)} = 17.5$, $P<0.001$). For those within the short and thin group, the association between healthy diet and ALP did not reach statistically significance. Corresponding associations in women did not reach statistically significance. Therefore, the second hypothesis could be accepted for men, but

not for women. However, early nutrition in women may not influence the relation between diet and bone health or the assumption that body size is a marker of early nutrition is not true in women. Thus, the second hypothesis of the study could not be rejected or accepted in women.

Using the same model for men and women, associations between dietary patterns with ALP and handgrip were constantly stronger in men than women, and thus, the third hypothesis should be accepted.

The findings of this study suggested that there was an added benefit of the dietary pattern, above and beyond that seen of the nutrients included in this analysis. It is not clear from the analyses undertaken in this thesis what the critical components of a healthy diet are for bone health; all that can be said is that by examining the pattern of diet provides additional information that can not be as yet identified by a nutrient analysis. The pattern analysis, therefore, can be referred to as a valuable tool for nutritional strategies in the context of public health nutrition. From a policy perspective, an approach that describes dietary patterns is most helpful because people do not eat nutrients and therefore any messages are most easily understood expressed as foods, rather than nutrients. In addition we do not as yet know the optimal combination of nutrients to maximize bone health. This suggests that attempts to improve bone health may be more successful if they emphasis dietary patterns rather than nutrients. It is therefore important to be clear what is the best dietary advice for the elderly, not just for bone health, but overall health.

The results provided support for the healthy dietary guidelines in the UK as being beneficial for bone health and muscle function in the elderly. However, according to the findings of this study, "healthy diet" would not be of benefit for some subgroups of the population. In general, none of eating patterns could be considered as a healthy diet for all subgroups of the population. In this study, body size was selected to stratify the population, because it was found that body size may act as an effect modifier, but it may also be possible to consider some other characteristics to target population groups.

Using the most interested eating pattern, only 2% of people from institutions and 14% of community dwelling people in the UK could be considered as healthy eaters. If the

healthy diet is important for bone health and muscle performance, policies are needed to encourage people to eat a more foods from this healthy pattern. However, in some sub-groups, such as institutionalised people the healthy diet was not associated with better bone health and stronger handgrip strength, calling for further investigation to determine the best dietary pattern for them and setting appropriate strategies aimed at improving their health status. If an older person is malnourished, the first priority should be to ensure that they are getting enough energy and the dietary pattern associated with increased energy intake may be most 'healthy' for that person. It is therefore very important to make sure that the right advice is given to the right people.

The differences in results by gender may suggest important hormone related effects that need to be explored further. From a public health point of view the concern is whether the dietary advice should be different in men and women? The measure of past growth and early life events used in this study was height and weight, which are at best crude markers of the exposure of interest. They do suggest, together with other research, that early life events may have long-term effects on bone health. The public health implications of this are that it may be important to target people with different body composition and to tailor dietary advice somewhat differently.

However, as with other studies, there are possible limitations that may affect the results and the conclusions of this study. Firstly, the outcome measure in this study was bone health, which is related to the effects of factors over the life period and therefore cross-sectional associations between current diet and bone health may not indicate causal relationship. Furthermore, because of the time lag between the diet and its effect on the development of a chronic condition such as osteoporosis, current diet may have limited relationship with present disease incidence, even though the associations are strong and statistically significant. Additionally, ALP is one of the markers of bone metabolism, which may not represent precisely the bone strength. Secondly, identified dietary patterns are specific to the study population and therefore, the results may not be generalisable to other populations. Similar analyses in different populations may be needed, particularly in different age groups to confirm the results. Thirdly, the dietary patterns described here only accounted for about 27% of the variation in food consumption, indicating the poten-

tial for other undetected patterns or dietary factors that may have affected ALP and hand-grip strength.

Summing up, this study showed that a diet, which complies with the UK healthy dietary guidelines, is positively associated with bone health and muscle performance in older population in the UK. The study showed the value of pattern analysis in nutritional studies and suggested the possible importance of considering dietary patterns instead of single nutrients in nutritional policies in the context of public health nutrition. Presented analyses showed how sex, body build and possibly early nutrition might interact with diet and bone health in the elderly.

The key massages of this study can be restated as below:

- The dietary pattern as a whole is more important than isolated nutrients or specific foods with respect to bone health and muscle function among older people.
- A healthy diet, as defined by the national dietary guidelines in the UK is associated with better bone health and stronger muscle performance in the elderly.
- UK healthy dietary guidelines seems to be effective for bone health and for the prevention of fall in general older population.
- Association between dietary patterns and bone health may vary for different population subgroups and body size may be used to target the subgroups in the population. Each group may need to be advised specifically.

Suggestion for future research:

Although studies of isolated nutrients should obviously continue, much attention should also be paid to food patterns as packages of foods and nutrients. An appropriate study design would be to feed a diet based on a food pattern and then observe the effects. Such a design automatically incorporates all nutrient interactions in the food pattern. Dropping certain foods from the patterns using a top-down logic approach will show the most beneficial combination of foods (dietary pattern).

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Appendix 1

Table. 1- Sex, age, household composition and social class based on occupation among responding sample of NDNS[1] and census 1991

	Responding sample			*1991 census data*		
	Men	Women	All	Men	Women	All
Age groups	%	%	%	%	%	%
65-74	66	51	57	64	55	58
75-84	29	37	34	31	36	34
85+	5	12	9	5	9	8
Total	40%	60%		41%	59%	
Living Alone	24	51	40	20	47	36
Living with others	76	49	60	80	53	64
Married	72	36	50	72	37	51
White Ethnicity	98	99	99	98	99	99
Social class of the household						
I&II	-	-	30	-	-	32
III N(Non-Manual)	-	-	9	-	-	13
III M (Manual)	-	-	37	-	-	28
IV and V	-	-	20	-	-	23
Others	-	-	3	-	-	3

[1] Free-living sample

Table. 2- Sex, age, household composition and social class based on occupation among responding and non-responding samples of NDNS

	Responding sample			*Non-Responding sample*		
	Men	Women	All	Men	Women	All
Age groups	%	%	%	%	%	%
65-74	60	41	48	66	33	43
75-84	31	35	34	26	46	40
85+	10	23	18	8	20	17
Total	37	62		31	69	
Mean age	74	78	76	73	78	77
Living Alone	26	59	43	30	65	53
Living with others	74	41	57	70	35	47
Married	74	39	54	62	24	36
White Ethnicity	99	99	99	98	98	98
Social class of the household						
I&II	-	-	31	-	-	23
III N(Non-Manual)	-	-	15	-	-	20
III M (Manual)	-	-	31	-	-	27
IV and V	-	-	22	-	-	29
Others	-	-	1	-	-	1

Appendix 2

Additional tables from chapter 6: results of analyses for BMI, weight, height with lifestyle and background variables.

Table 1- Spearman's correlation coefficient between BMI, body weight and height with dietary energy intake, ALP and total activity score. P values are 2-tailed.

	Energy intake	total activity score	Age	Household social class	ALK-P
BMI	.016	.039	-.137	.048	-.030
P value	.572	.145	.000	.117	.337
Weight	.267	.199	-.330	-.055	-.101
P value	.000	.000	.000	.073	.001
Height	.431	.243	-.348	-.141	-.103
P value	.000	.000	.000	.000	.001

Table 2-Distribution of BMI, weight and height by lifestyle variables.

Lifestyle variable	**BMI**	**Weight (kg)**	**Height(cm)**
Smoking	Mean	Mean	Mean
Smokers	24.9	65.1	162.2
Ex-smokers	26.6	71.4	163.8
Never-smokers	26.2	65.4	157.9
Long illness			
Yes	26.1	67.8	161.2
No	26.2	68.2	161.0
Domicile			
Free-living	26.4	69.4	162.0
Institution	24.9	61.2	157.5
Activity groups			
Low active	26.4	67.2	159.5
Moderate active	26.6	69.4	161.5
Very active	25.61	68.41	163.01

Table 3- Levels of bone affecting variables by third of height (NDNS)

		Third of height		
		Lowest third	Medium	Highest third
Household social class				
Class I and II	Count	67	107	128
	% within height third	24.3%	31.8%	37.4%
Class III non manual	Count	36	62	61
	% within height third	13.0%	18.4%	17.8%
Class III manual	Count	97	102	91
	% within height third	35.1%	30.3%	26.6%
Class IV and V	Count	76	66	62
	% within height third	27.5%	19.6%	18.1%
Total	Count	276	337	342
	% within height third	100.0%	100.0%	100.0%
Activity groups				
Low active	Count	142	107	88
	% within height third	37.3%	27.3%	22.6%
Moderate active	Count	124	150	119
	% within height third	32.5%	38.3%	30.5%
Very active	Count	115	135	183
	% within height third	30.2%	34.4%	46.9%
Total	Count	381	392	390
	% within height third	100.0%	100.0%	100.0%
Smoking habit				
Never-smoker	Count	168	149	145
	% within height third	43.4%	38.0%	37.3%
Ex-smoker	Count	157	180	186
	% within height third	40.6%	45.9%	47.8%
Smoker	Count	62	63	58
	% within height third	16.0%	16.1%	14.9%
Total	Count	387	392	389
	% within height third	100.0%	100.0%	100.0%
Domicile				
Free-living	Count	294	358	358
	% within height thirds	76.0%	91.3%	91.8%
Institution	Count	93	34	32
	% within height thirds	24.0%	8.7%	8.2%
Total	Count	387	392	390
	% within height thirds	100.0%	100.0%	100.0%
Age groups				
65-74	Count	107	172	202
	% within height thirds	27.6%	43.9%	51.8%
75-84	Count	162	150	142
	% within height thirds	41.9%	38.3%	36.4%
85+	Count	118	70	46
	% within height thirds	30.5%	17.9%	11.8%
Total	Count	387	392	390
	% within height thirds	100.0%	100.0%	100.0%
Long illness				
Yes	Count	262	267	275
	% within height thirds	67.7%	68.3%	70.5%
No	Count	125	124	115
	% within height thirds	32.3%	31.7%	29.5%
	Count	387	391	390
	% within height thirds	100.0%	100.0%	100.0%

Table 4-Levels of bone affecting variables by third of weight (NDNS)

		Thirds of weight		
		Lowest third	Medium	Highest third
Household social class				
Class I & II	Count	83	111	113
	% within weight third	29.0%	31.3%	31.7%
Class III non manual	Count	49	65	55
	% within weight third	17.1%	18.3%	15.4%
Class III manual	Count	83	109	103
	% within weight third	29.0%	30.7%	28.9%
Class IV and V	Count	71	70	85
	% within weight third	24.8%	19.7%	23.9%
Total	Count	286	355	356
	% within weight third	100.0%	100.0%	100.0%
Activity groups				
Low active	Count	140	119	129
	% within weight third	33.7%	28.0%	30.9%
Moderate active	Count	131	132	157
	% within weight third	31.5%	31.1%	37.6%
Very active	Count	145	174	131
	% within weight third	34.9%	40.9%	31.4%
Total	Count	416	425	417
	% within weight third	100.0%	100.0%	100.0%
Smoking habit				
Never-smoker	Count	184	158	172
	% within weight third	44.0%	37.3%	41.1%
Ex-smoker	Count	154	205	195
	% within weight third	36.8%	48.3%	46.7%
Smoker	Count	80	61	51
	% within weight third	19.1%	14.4%	12.2%
Total	Count	418	424	418
	% within weight third	100.0%	100.0%	100.0%
Domicile				
Free-living	Count	303	370	380
	% within weight third	72.0%	87.1%	90.9%
Institution	Count	118	55	38
	% within weight third	28.0%	12.9%	9.1%
Total	Count	421	425	418
	% within weight third	100.0%	100.0%	100.0%
Age groups				
65-74	Count	99	172	210
	% within weight third	23.5%	40.5%	50.2%
75-84	Count	167	165	153
	% within weight third	39.7%	38.8%	36.6%
85+	Count	155	88	55
	% within weight third	36.8%	20.7%	13.2%
Total	Count	421	425	418
	% within weight third	100.0%	100.0%	100.0%
Long illness				
Yes	Count	300	299	284
	% within for weight	71.4%	70.4%	67.9%
No	Count	120	126	134
	% within for weight	28.6%	29.6%	32.1%
Total	Count	420	425	418
	% within for weight	100.0%	100.0%	100.0%

Table 5- Univariate analysis of the interaction of thirds of weight and height on healthy dietary score, controlling for energy intake in men and women (NDNS) dependent Variable: healthy diet score.

Source	df	Mean Square	F	Sig.
Men				
Corrected model	9	8.948	7.592	.000
Energy intake	1	38.190	32.403	.000
Thirds of weight	2	1.125	.955	.386
Thirds of height	2	6.819	5.786	.003
Error	577	1.179		
Women				
Corrected model	9	3.772	4.361	.000
Energy intake	1	1.344	1.554	.213
Thirds of weight	2	4.488	5.189	.006
Thirds of height	2	5.705	6.595	.001
Error	530	.865		

Table 6- Univariate analysis of the interaction of thirds of weight and height on ALP, controlling for energy intake in men and women (NDNS) dependent Variable: logarithmically transformed value of ALP.

Source	df	Mean Square	F	Sig.
Men				
Corrected model	8	.047	2.115	.033
Thirds of weight	2	.074	3.373	.035
Thirds of height	2	.026	1.157	.315
Error	458	.022		
Women				
Corrected model	8	.016	.837	.570
Thirds of weight	2	.008	.403	.669
Thirds of height	2	.015	.795	.452
Error	401	.019		

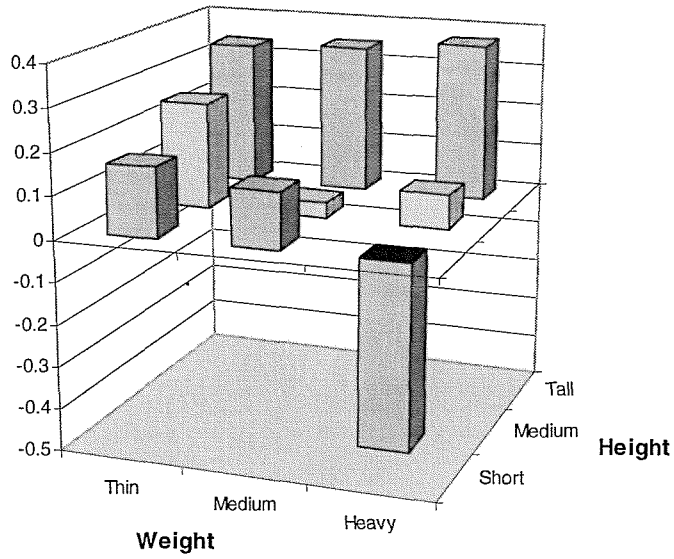

Figure 1- Distribution of healthy dietary score by w/h thirds in men after controlling for energy intake, BMI and social class, using univariate ANOVA. Bar charts represent the means of healthy diet scores in each group after controlling for other variables.

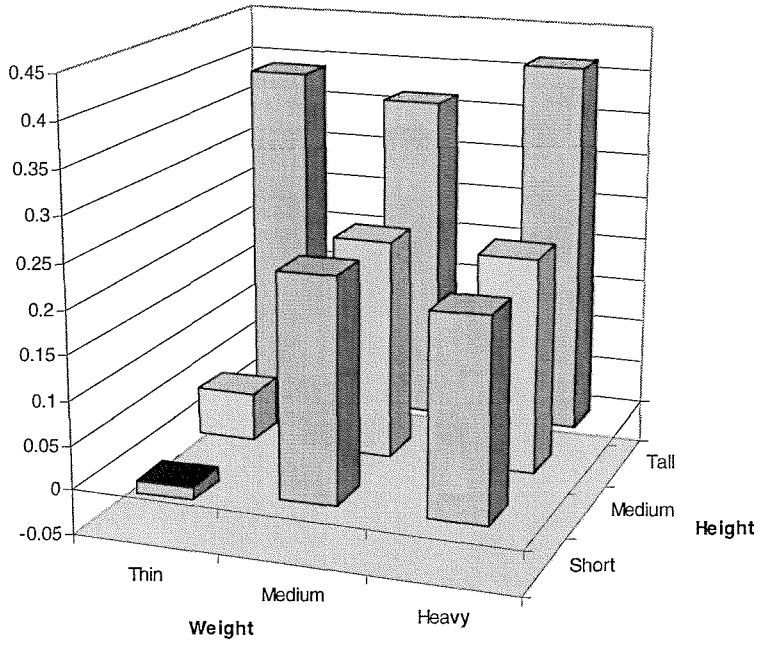

Figure 2- Distribution of healthy dietary score by w/h thirds in women after controlling for energy intake, BMI and social class, using univariate ANOVA. Bar charts represent the means of healthy diet scores in each group after controlling for other variables.

		Thin short	Thin medium	Thin tall	Medium short	Medium medium	Medium tall	Heavy short	Heavy medium	Heavy tall
Social class Iand II	Count	34	35	10	24	37	46	9	32	69
	%within w/h[1]	27.0%	43.2%	21.3%	25.5%	28.9%	40.4%	17.6%	28.3%	39.7%
IIIN	Count	20	9	12	9	26	26	7	23	23
	% within w/h	15.9%	11.1%	25.5%	9.6%	20.3%	22.8%	13.7%	20.4%	13.2%
IIIM	Count	42	21	11	34	44	26	18	30	52
	% within w/h	33.3%	25.9%	23.4%	36.2%	34.4%	22.8%	35.3%	26.5%	29.9%
IV and V	Count	30	16	14	27	21	16	17	28	30
	% within w/h	23.8%	19.8%	29.8%	28.7%	16.4%	14.0%	33.3%	24.8%	17.2%
Physical activity										
Low active	Count	65	25	15	40	38	27	30	38	44
	% within w/h	35.3%	24.3%	27.3%	34.2%	26.2%	20.6%	44.8%	30.2%	22.6%
Moderate active	Count	56	41	14	42	46	31	22	54	70
	% within w/h	30.4%	39.8%	25.5%	35.9%	31.7%	23.7%	32.8%	42.9%	35.9%
Very active	Count	63	37	26	35	61	73	15	34	81
	% within w/h	34.2%	35.9%	47.3%	29.9%	42.1%	55.7%	22.4%	27.0%	41.5%
Domicile Free-living	Count	135	88	47	96	135	119	57	120	185
	% within w/h	72.2%	85.4%	85.5%	82.1%	93.1%	90.8%	83.8%	95.2%	94.9%
Institution	Count	52	15	8	21	10	12	11	6	10
	% within w/h	27.8%	14.6%	14.5%	17.9%	6.9%	9.2%	16.2%	4.8%	5.1%
Age groups 65-74	Count	39	35	22	35	66	68	30	63	108
	% within w/h	20.9%	34.0%	40.0%	29.9%	45.5%	51.9%	44.1%	50.0%	55.4%
75-84	Count	76	38	26	55	54	42	26	51	69
	% within w/h	40.6%	36.9%	47.3%	47.0%	37.2%	32.1%	38.2%	40.5%	35.4%
85+	Count	72	30	7	27	25	21	12	12	18
	% within w/h	38.5%	29.1%	12.7%	23.1%	17.2%	16.0%	17.6%	9.5%	9.2%
Long illness yes	Count	122	70	42	85	98	93	41	86	135
	% within w/h	65.2%	68.6%	76.4%	72.6%	67.6%	71.0%	60.3%	68.3%	69.2%
no	Count	65	32	13	32	47	38	27	40	60
	% within w/h	34.8%	31.4%	23.6%	27.4%	32.4%	29.0%	39.7%	31.7%	30.8%

[1] Percents are within each weight and height groups

Table 7-Characteristics of weight and height groups in terms of lifestyle and background variables (NDNS).