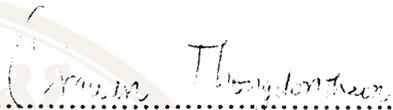


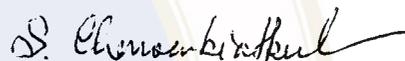


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AFFECTING BONE STATUS IN HEALTHY THAI CHILDREN  
AGED 9-12 YEARS**



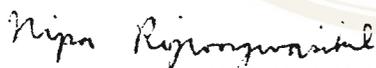
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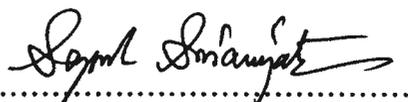
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was submitted to the Faculty of Graduate Studies, Mahidol University for the degree of  
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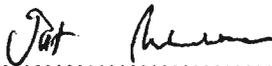
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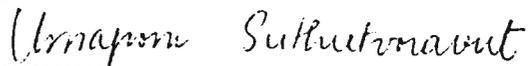
  
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KEY WORDS : DIETARY CALCIUM / PHYSICAL ACTIVITY / PHYSIOLOGICAL FACTORS / BONE STATUS / DUAL ENERGY X-RAY ABSORPTIOMETRY / CHILDREN AND ADOLESCENTS

ORAWAN THONGDONTAON : DIETARY CALCIUM INTAKE AND OTHER FACTORS AFFECTING BONE STATUS IN HEALTHY THAI CHILDREN AGED 9-12 YEARS. THESIS ADVISORS : SOMSRI CHAROENKIATKUL, D.Sc. VONGSAVAT KOSULWAT, Ph.D. UMAPORN SUTHUTVORAVUT, M.D. NIPA ROJROONGWASINKUL, M.Sc. 184 p. ISBN 974-663-736-3

Osteoporosis is characterized by low bone mass and micro architectural deterioration of bony tissue, leading to high risk fracture. Maximizing peak bone mass during growth constitutes one of the best preventive strategies. This approach requires better understanding of bone acquisition and its contributing factors. Therefore, this study was conducted to investigate dietary calcium and other factors affecting bone mass in 274 healthy Thai children and adolescents from Samsen and Phayathai schools, aged 9-12 years. Bone mineral content (BMC, g) and areal bone mineral density (BMD,  $\text{gm.cm}^{-2}$ ) were measured by dual energy x-ray absorptiometry (DEXA) at midshaft radius, lumbar spine (L2-4), and femoral neck (FN), including total body BMD, total body BMC and total body calcium (TBC). Calculated volumetric bone mineral density (vBMD,  $\text{gm.cm}^{-3}$ ) was also evaluated. Current dietary intake was assessed by 3-day food record whereas usual calcium intake was estimated by semi-quantitative food frequency questionnaire. Physical activity was measured by self-administered questionnaire.

Dietary assessment revealed that there was no significant difference in calcium intake at any age group, pubertal stage as well as between sex with mean intake of  $511 \pm 230 \text{ mg.d}^{-1}$ . Mean time spent for weight bearing activity was  $6.5 \pm 5.8 \text{ hr.wk}^{-1}$  with significant difference between males and females. The BMD values at all sites tended to increase with advancing age and the values reach statistical significant difference from age 11 to 12 years old in both genders except for FNBMD in males. No significant increment of vBMD at L2-4 spine and FN site was observed in both males and females except for the value at lumbar spine in females which observed a significant change starting from age 11 years. A significant gender effect was observed among subjects who had the same age especially on the areal BMD and TBC in the 11-year old group except for FN. Males in younger age group had significant higher BMD at midshaft radius and FN and BMC at FN than females. Significant effect of pubertal status on bone change at a variety of bone sites was observed by exhibiting higher bone values among pubertal children than those in pre-pubertal stage. The number of hours spent for weight bearing activity on a weekly basis also had a significant effect of bone increment especially the BMD value at different bone sites in male subjects. Results from multiple regression analysis confirmed the findings by demonstrating that age, sex, puberty, weight for height were the major determinants of bone mass, accounting for 24-79% of the variability. Usual calcium intake was environmental factor which determined bone mass especially at midshaft radius in both genders. Weight bearing activity appeared to be another independent determinant, describe the variability on total body and at lumbar spine.

In conclusion, physiological factors were the major determinant on bone mass. Calcium intake and weight bearing activity were associated with bone mass at different sites especially in subjects whose usual calcium intake and time spent for weight bearing activity were above their mean. Therefore, maximizing peak bone mass can be achieved in these children with proper weight, adequate calcium intake and moderate physical activity during the optimum time of bone growth.

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โรคกระดูกเป็นภาวะที่มวลกระดูกลดลง เป็นผลทำให้กระดูกเปราะบาง และเสี่ยงต่อการหักของกระดูก โรคนี้กำลังเป็นปัญหาสาธารณสุขและสังคมเศรษฐกิจที่สำคัญปัญหาหนึ่งทั่วโลก การเพิ่มมวลกระดูกให้ได้ถึงจุดสูงสุด ถือว่าเป็นการป้องกันโรคกระดูกพรุนที่มีประสิทธิภาพมากที่สุด อย่างไรก็ตามการศึกษาเกี่ยวกับปัจจัยที่มีผลต่อมวลกระดูก ยังไม่เคยได้ทำการศึกษาในเด็กมาก่อน การเข้าใจถึงลักษณะการเพิ่มของมวลกระดูก เป็นสิ่งจำเป็นที่จะทำให้ป้องกันภาวะโรคกระดูกพรุนได้ การศึกษาครั้งนี้เป็นการศึกษากภาคตัดขวาง (cross-sectional study) กลุ่มตัวอย่างเป็นเด็กและวัยรุ่นที่มีอายุระหว่าง 9-12 ปี จำนวน 274 คน โดยดำเนินการวัดความหนาแน่นของกระดูก (bone mineral density, BMD) และปริมาณแร่ธาตุในกระดูก (bone mineral content, BMC) ของกระดูกทั่วร่างกาย (total body) และตำแหน่งที่สำคัญ เช่น กระดูกกึ่งกลางแขน (midshaft radius) กระดูกสันหลังระดับเอว (lumbar spine) กระดูกต้นขา (femoral neck) รวมทั้งปริมาณแคลเซียมทั่วร่างกาย (total body calcium, TBC) และคำนวณความหนาแน่นกระดูกต่อปริมาตร (volumetric bone mineral density, vBMD) ในบริเวณกระดูกสันหลังระดับเอวและกระดูกต้นขา การประเมินการบริโภคอาหาร ใช้วิธีการบันทึกอาหารและแบบสอบถามความถี่การบริโภคอาหาร ข้อมูลทั่วไปและการประเมินการออกกำลังกายโดยใช้แบบสอบถาม

ผลการศึกษาพบว่า เด็กไทยได้รับแคลเซียมจากอาหารเฉลี่ย 511 มก.ต่อวัน โดยไม่พบความแตกต่างระหว่างกลุ่มอายุ เพศ และการเข้าสู่วัยรุ่น และใช้ระยะเวลาในการออกกำลังกายแบบรับน้ำหนักเฉลี่ย 6.5 ชม.ต่อสัปดาห์ ไม่พบความแตกต่างของเวลาที่ใช้ในการออกกำลังกายในแต่ละกลุ่มอายุ แต่พบว่าเด็กผู้ชายใช้เวลาในการออกกำลังกายแบบรับน้ำหนักมากกว่าเด็กหญิงที่อายุเท่ากัน ความหนาแน่นของกระดูกเพิ่มขึ้นตามอายุที่เพิ่มมากขึ้นทั้งในเด็กชายและหญิงอย่างมีนัยสำคัญทางสถิติเมื่ออายุ 11-12 ปี แต่ไม่พบการเปลี่ยนแปลงของกระดูกต้นขาตามอายุที่เพิ่มมากขึ้นในเด็กผู้ชาย ส่วนความหนาแน่นของกระดูกต่อปริมาตรพบการเปลี่ยนแปลงตามอายุที่เพิ่มมากขึ้นเฉพาะในเด็กหญิงอย่างมีนัยสำคัญทางสถิติที่อายุ 11 ปี เด็กชายมีความหนาแน่นของกระดูกต้นขามากกว่าเด็กหญิงอย่างมีนัยสำคัญทางสถิติเมื่ออายุ 9 ปี และพบว่าปริมาณแร่ธาตุในกระดูกต้นขายังคงมากกว่าเด็กหญิงเมื่ออายุ 10 ปี ในขณะที่เด็กหญิงมีความหนาแน่นของกระดูกและปริมาณแร่ธาตุในกระดูกมากกว่าเด็กชายเมื่ออายุ 11 ปี เมื่อเข้าสู่วัยรุ่นเด็กชายและหญิงจะมีความหนาแน่นกระดูกทั่วร่างกายและตำแหน่งต่างๆของกระดูกมากกว่าระยะก่อนเข้าสู่วัยรุ่น ระยะเวลาที่ใช้ในการออกกำลังกายแบบรับน้ำหนักที่มากกว่าค่าเฉลี่ยของกลุ่ม (>6.5 ชม.ต่อสัปดาห์) มีผลทำให้เกิดความหนาแน่นกระดูกทั่วร่างกายบริเวณกระดูกสันหลังระดับเอวและกระดูกต้นขามากกว่าเด็กที่ใช้เวลาในการออกกำลังกายน้อยกว่า เมื่อพิจารณาผลของการวิเคราะห์การถดถอยพหุคูณพบว่าอายุ เพศ การเข้าสู่วัยรุ่น และน้ำหนักต่อส่วนสูง เป็นปัจจัยสำคัญที่มีผลทำให้เกิดความแปรปรวน 24-79% ของความแปรปรวนที่เกิดขึ้นในมวลกระดูก แคลเซียมจากอาหารสามารถอธิบายความแปรปรวนที่เกิดขึ้นในกระดูกกึ่งกลางแขน ในขณะที่การออกกำลังกายแบบรับน้ำหนักมีผลต่อมวลกระดูกทั่วร่างกายและกระดูกสันหลังระดับเอว

การศึกษานี้สรุปได้ว่า ปัจจัยทางด้านสรีรภาพ ได้แก่ อายุ เพศ การเข้าสู่วัยรุ่น และน้ำหนักต่อส่วนสูงมีผลต่อมวลกระดูกทั่วร่างกายและตำแหน่งต่างๆ แคลเซียมจากอาหารและการออกกำลังกายแบบรับน้ำหนักมีผลต่อมวลกระดูกในบางตำแหน่งนอกจากนี้ยังพบว่าแคลเซียมจากอาหารและการออกกำลังกายแบบรับน้ำหนักจะมีผลต่อกระดูกก็ต่อเมื่อมีการบริโภคแคลเซียมและใช้เวลาในการออกกำลังกายมากกว่าค่าเฉลี่ยของกลุ่ม ซึ่งจะเห็นได้ว่าการเพิ่มมวลกระดูกสูงสุดสามารถกระทำได้ โดยการส่งเสริมให้เด็กไทยมีน้ำหนักตัวที่เหมาะสมกับส่วนสูง บริโภคแคลเซียมอย่างเพียงพอและใช้เวลาในการออกกำลังกายแบบรับน้ำหนักอย่างสม่ำเสมอ ซึ่งการเพิ่มมวลกระดูกสูงสุดดังกล่าว จะสามารถเพิ่มได้มากในช่วงวัยเด็กและวัยรุ่น

# CONTENTS

	page
<b>ACKNOWLEDGEMENT</b>	<b>iii</b>
<b>ABSTRACT</b>	<b>iv</b>
<b>LIST OF CONTENTS</b>	<b>vi</b>
<b>LIST OF TABLES</b>	<b>viii</b>
<b>LIST OF FIGURES</b>	<b>xi</b>
<b>LIST OF APPENDIXS</b>	<b>xiii</b>
<b>LIST OF ABBREVIATIONS</b>	<b>xiv</b>
<b>CHAPTER</b>	
<b>I INTRODUCTION</b>	<b>1</b>
<b>II LITERATURE REVIEW</b>	<b>6</b>
2.1 Overview of bone	6
2.2 Type of bone	7
2.3 Bone acquisition in childhood and adolescence	9
2.4 Factors affecting bone mineral density	15
2.4.1 Genetic/Ethnic determinants	16
2.4.2 Age and gender	18
2.4.3 Puberty	19
2.4.4 Dietary factor	20
2.4.5 Physical activity	27
2.4.6 Body Mass	28
2.5 Bone status measurement	29
2.6 Osteoporosis	31
<b>III MATERIALS AND METHODS</b>	<b>33</b>
3.1 Subjects of study subjects	33
3.2 Overview of study design	34
3.3 Study site	34
3.4 Methods of data collection	34
3.4.1 Dietary assessment	34
3.4.2 Bone mineral measurement	36

## CONTENTS (continue)

	<b>page</b>
3.4.3 Physical examination and sexual maturation assessment	42
3.4.4 Anthropometric assessment	42
3.4.5 Physical activity	43
3.5 Data analysis	43
3.5.1 Dietary data analysis	43
3.5.2 The validation study	44
3.5.3 Statistical analysis	50
<b>IV RESULTS</b>	<b>54</b>
4.1 Demographic characteristics of the study subjects	54
4.2 Daily dietary and nutrient intake	57
4.3 Factors affecting bone mass	67
4.3.1 Age and gender	67
4.3.2 Puberty	70
4.3.3 Additive effect of usual dietary calcium intake	76
4.3.4 Weight bearing activity	79
4.4 Relationship between different variables on bone parameters	84
4.5 Multiple regression analysis	94
<b>V DISCUSSION</b>	<b>99</b>
<b>VI CONCLUSION</b>	<b>113</b>
<b>REFERENCES</b>	<b>117</b>
<b>BIOGRAPHY</b>	

## LIST OF TABLES

<b>Table</b>	<b>page</b>
3.1 Estimates of group mean dietary calcium intake of children subjects, obtained from 6-three day food records and semi-quantitative FFQ	48
3.2 Cross-classification of dietary calcium intake of children subjects from 3-day food record and SFFQ	48
4.1 Demographic characteristics of the subjects classified by sex	55
4.2 Anthropometric parameters of the subjects classified by sex	56
4.3 Mean $\pm$ SD of daily dietary intake from 3-day food record of the subjects classified sex	58
4.4 Mean $\pm$ SD of calcium, phosphorus, protein, and sodium intake from 3-day food record of the subject according to sex and age group	59
4.5 Mean $\pm$ SD of daily dietary calcium, phosphorus, protein, and sodium intake from 3-day food record of the subjects classified by sex and pubertal stage	60
4.6 Number and percentage distribution of the subjects classified by sex and level of dietary calcium intake from 3-day food record according to the RDA recommendation	62
4.7 Number and percentage distribution of the subjects classified by sex and level of dietary calcium intake from semiquantitative food frequency questionnaire according to the RDA recommendation	62
4.8 Mean $\pm$ SD and the percentage contribution of food group of daily dietary calcium intake of subjects according to sex	64
4.9 Mean $\pm$ SD of real bone mineral density and volumetric bone mineral and volumetric BMD at midshaft radius, lumbar spine, femoral neck and total body of subjects according to sex and age group	66

## LIST OF TABLES (continue)

<b>Table</b>	<b>page</b>
4.10 Mean $\pm$ SD of bone mineral content at midshaft radius, lumber spine, femoral neck, and total body calcium of subjects according to sex and age group	68
4.11 Mean $\pm$ SD of areal bone mineral density and volumetric bone mineral density at midshaft radius, lumber spine, femoral neck and total body calcium of subjects classified by sex and pubertal stage	71
4.12 Mean $\pm$ SD of bone mineral content at midshaft radius, lumber spine, femoral neck, total body BMC and total body calcium of subjects classified by sex and pubertal stage	74
4.13 Mean $\pm$ SD of bone mineral density classified by level of calcium intake from FFQ, sex and pubertal stage of subject	77
4.14 Mean $\pm$ SD of bone mineral content classified by level of calcium intake from FFQ, sex and pubertal stage of subject	78
4.15 Mean $\pm$ SD of time spent for weight bearing activity of the subjects according to sex and age group	80
4.16 Mean $\pm$ SD of bone mineral density classified by sex and level of activity of subjects	81
4.17 Mean $\pm$ SD of bone mineral content classified by level of activity and sex of subjects	82

**LIST OF TABLES (continue)**

<b>Table</b>	<b>page</b>
4.18 Correlation coefficient between various variables and bone mineral density of the subjects	92
4.19 Correlation coefficient between various variables and bone mineral content of the subjects	93
4.20 Multiple regression analysis of various factors on bone mineral density	96
4.21 Multiple regression analysis of various factors on bone mineral content	98

## LIST OF FIGURES

Figure	page
1. Boxplot of areal bone mineral density ( $\text{g.cm}^{-2}$ ) and volumetric bone mineral density ( $\text{g.cm}^{-3}$ ) against age for males and females at midshaft radius, lumbar spine, femoral neck and total body	67
2. Boxplot of bone mineral content (g) against age for male and females at midshaft radius, lumbar spine, femoral neck, total body and total body calcium	69
3. Mean $\pm$ SD bone mineral density ( $\text{g.cm}^{-2}$ ) and volumetric bone mineral density ( $\text{g.cm}^{-3}$ ) for males and females at midshaft radius, lumbar spine, femoral neck and total body according to sex and pubertal stage	72
4. Mean $\pm$ SD bone mineral content (g) for males and females at midshaft radius, lumbar spine, femoral neck, total body BMC and total body calcium according to sex and pubertal stage	75
5. Relationship between age and bone mineral density ( $\text{g.cm}^{-2}$ ) at midshaft radius, lumbar spine, femoral neck and total body of the subjects	85
6. Relationship between age and bone mineral content (g) at midshaft radius, lumbar spine, femoral neck, total body BMC and total body calcium of the subjects	86
7. Relationship between dietary calcium intake and bone mineral density ( $\text{g.cm}^{-2}$ ) at midshaft radius and bone mineral content (g) at midshaft of radius and femoral neck of the subjects	89

## LIST OF FIGURES (continus)

<b>Figure</b>	<b>page</b>
8. Relationship between weight bearing activity and bone mineral density (g.cm <sup>-2</sup> ) at lumbar spine, femoral neck and total body of the subjects	90
9. Relationship between weight bearing activity and bone mineral content (g) at midshaft radius, lumbar spine, femoral neck, total body BMC and total body calcium of the subjects	91
10. Boxplot of bone mineral density (g.cm <sup>-2</sup> ) at midshaft radius and lumbar spine, total body BMC (g) and total body calcium (g) against dietary calcium intake in male subjects	107
11. Boxplot of bone mineral density (g.cm <sup>-2</sup> ) at midshaft radius and lumbar spine, total body BMC (g) and total body calcium (g) against dietary calcium intake in female subjects	108
12. Boxplot of bone mineral density (g.cm <sup>-2</sup> ) at midshaft radius and lumbar spine, total body BMC (g) and total body calcium (g) against time spent for weight bearing activity in male subjects	110
13. Boxplot of bone mineral density (g.cm <sup>-2</sup> ) at midshaft radius and lumbar spine, total body BMC (g) and total body calcium (g) against time spent for weight bearing activity in female subjects.	111

## LIST OF APPENDIXS

<b>Appendix</b>	<b>page</b>
A Puberty	134
B Semiquantitative food frequency questionnaire	136
C Physical activity and exercise questionnaire	151
D Sport activity	155
E.1 Mean $\pm$ SD of bone size of the subjects classified by sex and pubertal stage	156
E.2 Mean $\pm$ SD of bone size of the subjects classified by sex and age group	157
E.3 Correlation coefficient between various variables and bone parameter of male subjects	158
E.4 Correlation coefficient between various variables and bone parameter of female subjects	159
F.1 Mean $\pm$ SD of BMD at lumbar spine and femoral neck of the subjects comparing with young adult	160
F.2 Mean $\pm$ SD of total body bone mass of Thai children comparing with reference data	161
G Multiple regression analysis of various factors on bone value	162

## LIST OF ABBREVIATIONS

aBMD	=	areal bone mineral density
BMC	=	bone mineral content
BMD	=	bone mineral density
Ca	=	calcium
cm	=	centimeter
d	=	day
DEXA	=	dual energy x-ray absorptiometry
F	=	female
Fig.	=	figure
FR	=	food record
g	=	gram
haz	=	height for age
hr	=	hour
ht	=	height
keV	=	kiloelectron voltage
M	=	male
mA	=	milliampere
mg	=	milligram
NCHS	=	National Center of Health Statistics
P	=	phosphorus
RDA	=	Recommended Dietary Allowances
SD	=	standard deviation
sFFQ	=	semiquantitative food frequency questionnaire
TBC	=	total body calcium
vBMD	=	volumetric bone mineral density
waz	=	weight for age
WBA	=	weight bearing activity
wk	=	week

wt	=	weight
y	=	year
QCT	=	quantitative computed tomography
kcal	=	kilocalorie



## CHAPTER I

### INTRODUCTION

#### 1.1 Rationale of the study

Osteoporosis has been considered as an important public health problem in many countries, in terms of morbidity, mortality and socioeconomic burden. It is characterized by low bone mineral density (BMD) and microarchitectural deterioration, leading to fracture in the hip, spine, distal forearm and other skeletal sites. These consequences results in longer period of hospitalization, disability, loss of independence, and the increment of medical cost of health services. The total medical expenditure for treatment and other services utilization for osteoporosis in the United States were estimated to be 13 billion dollars annually<sup>(1)</sup> and the cost of hip fracture alone may exceed 240 billion dollars by the year 2040.<sup>(2)</sup> Moreover, the fatality rate of hip fracture patient within 1 year following the fracture was approximately 20%.<sup>(3,4)</sup>

The prevalence of osteoporotic fracture is expected to increase dramatically because the population tend to have longer life expectancy following by an increment of aging population. There are somewhat in excess of 325 million individuals with age over 65 years globally and will increase to greater than 1,500 million by the year 2050.<sup>(5)</sup> According to projection by World Health Organization, there will be a total of 900 million men and women 65 years of age and older in Asia by the year 2050. In

1990, 30% of total hip fractures in the world were reported from Asian population and will reach to more than 50% by the year 2050, around 3.2 millions per year.<sup>(5)</sup> In Thailand, the life expectancy of Thai population is also increasing. The National Economic and Social Development Board (NESDB) has projected that life expectancy of the Thai people will increase from 48.5 and 51.4 years in 1947 to 66.8 and 70.8 years in 2000 AD, in men and women, respectively. The percentage of Thai people over 60 years old will be 7.5% in the year 2000. The incidence rate of osteoporotic fracture is therefore expected to increase and still rising with older age.<sup>(6)</sup> Suriyawongpaisal reported that the accumulated incidence rate of hip fracture per 100,000 population were 0.30 among patients aged 40-45 years and has risen to 15.19 and 61.18 in patients aged 70-74 and 75-95 years, respectively.<sup>(7)</sup> He also observed that an increase in the prevalence of hip fracture was inversely proportionate to the bone mineral density of Thai population. Since bone mineral density is an important determinant of bone status and also associated with incidence rate of osteoporotic fracture. Several studies attempted to determine factor (s) that influence the BMD, identifying the potential factors which could help to predict the risk of osteoporosis and develop an effective strategy for the prevention the development of osteoporosis.

Preventing osteoporosis is to maximize bone mass by enhancing factors which may contribute to it. Two major contributing factors of bone mineral density are genetic and environmental factors. Although it is unchangeable, genetic factor plays a major role in determining the bone status which accounting for approximately 70-80% of total variance.<sup>(8)</sup> Physiological factors such as age, sex, puberty, weight and height, which are also modulated by genetic factor, have been reported as association with

bone mass. Bone mass increases with advancing age and differs between genders and skeletal sites.<sup>(9)</sup> There are evidences indicating that bone mineral density (BMD) increases dramatically during puberty in both boys and girls.<sup>(10,11,12)</sup> Individuals with early onset of puberty and long reproductive period have higher BMD than those with late onset and short reproductive period.<sup>(13)</sup>

The environmental factors can modulate bone mass and bone density through calcium intake and physical activity. An adequate calcium intake can increase bone mass in children and adolescents.<sup>(8,14,15)</sup> On the contrary, low dietary calcium intake has adversely affected on bone mass.<sup>(16,17)</sup> Asian population consumed dietary calcium approximately 400-600 mg/day,<sup>(17,18,19,20)</sup> lower than the Recommended Dietary Allowance for Thai (RDA, 800 mg/day). For children, Lee et al.<sup>(15)</sup> reported that calcium intake of children and adolescents, in Hong Kong was about 300-400 mg/day and found an increase in BMD and bone width with calcium supplement. Johnston also reported that after three years supplementation of calcium in the form of calcium citrate-malate tablet (1600 mg/day) in white identical twin children who normally consumed dietary calcium at the same level as recommended by RDA resulting in greater bone mineral density in both radial site and lumbar spine than control group.<sup>(8)</sup> It is still unclear that how much calcium intake is optimum to promote bone growth and development among different population.

The optimal time to increase bone mass and density should be at childhood and adolescent period.<sup>(21,22)</sup> There were evidences suggested that peri-pubertal (midpuberty) child, starting as early as 10 years of age, seemed to be most responsive to modification of environmental and life style factors because of the maximal growth spurt and higher

rate of bone accretion in this age group.<sup>(23,24)</sup> During puberty, there was an increment of bone mass approximately 2.5 fold higher than pre-pubertal children.<sup>(25)</sup> Children who have moderate weight bearing activity also had higher bone mass than control group.<sup>(26)</sup>

Since there has never been any report on bone mass of Thai children and adolescence and Thai population especially the later aged group are now facing with the problem of osteoporosis. No preventive strategy for this disease has been setting up. In this study, we aimed to determine factor(s) influencing bone status in Thai children aged between 9-12 years. Furthermore, as it is widely accepted that the effective preventive strategies of osteoporosis can be easily made through the modification of environmental factors especially dietary calcium intake. We would like to know how much dietary calcium is usually consumed by Thai children. Not only the level of dietary calcium intake was the main focus of our study but also major food sources of calcium consumed by this population. The bone status in childhood and adolescent Thai subject was also determined in this study.

In summary, this work is considered as the first attempt to correlate the effect of several factors especially environmental factors on bone status in Thai children and adolescents by using a cross sectional study approach. The information derived from this study will serve as foundation in planning for future nutritional implementation program for the public as well as for planning of future research.

## **1.2 Objective**

1. To evaluate source and amount of calcium intake in Thai children subjects
2. To determine bone status in Thai children subjects

3. To determine relationship between physiological and environmental factors and bone mass of Thai children subjects.

### **1.3 The expected benefit of this study**

Since we have a very limited information of bone mass and its related factors in Thai population, this study will serve as an initial step for osteoporosis prevention. The result of this study will be useful for setting up an appropriate RDA of calcium recommendation for Thai children to promote bone health. Furthermore if one would like to propose dietary intervention strategy to promote calcium intake by using food-based approach, information derived from this study particularly the evaluation of dietary calcium intake and identification of food sources for calcium by which mostly consumed by Thai children and adolescents will contribute to an enhancement bone mineral density leading to maximizing peak bone mass, and prevention of osteoporosis in later life.

## CHAPTER II

### LITERATURE REVIEW

#### 2.1 Overview of bone

Bone can be defined at two levels. As a tissue, bone has two components, an organic and inorganic substance.<sup>(27)</sup> Forty percent of the bone is an organic substance and mainly cell and matrix. The function of matrix is a cement and fundamental structure. The major component of matrix are collagen while the other are glycoprotein, phospho-protein, and osteocalcin. Another component of bone is inorganic substance such as calcium and phosphorus, which together with water constitute a hydroxyapatite-like crystal with the general formula  $\text{Ca}_{10}\text{H}(\text{PO}_4)_6\text{OH}_2$  and amorphous calcium phosphate  $\text{Ca}_3(\text{PO}_4)_2$ , imparting structure and strength. Hydroxyapatite is deep within bone matrix, whereas amorphous calcium phosphate coat the surface of newly formed or remodeled bone. Bone salt is not pure calcium and phosphorus, it contains anions such as carbonate and citrate, and cations, such as magnesium (Mg) and zinc (Zn).<sup>(28)</sup>

As an organ, bone is a component of the skeleton. It is surrounded by periosteum, and composed of cortical and trabecular bone, cartilage, haematopoietic and connective tissue. They are all together the skeletal system, which has both biomechanical and metabolic functions. The hardness and rigidity of bone enable the skeleton to maintain the shape of the body, to protect the soft tissue, to provide a framework for the bone marrow and to transmit the force of muscular contraction from one part of the body to

another. Moreover, bone is also the major body reservoir for calcium, phosphorus, magnesium and carbonate which are component of many compound within and outside bone. It can be easily degraded and turnover whenever body needed. The degree of turnover rate is different among bone types.

## **2.2 Type of bone**

The skeleton consists of two types of bone. Most bones of the skeleton arise from secondary transformation of cartilage and are referred to as enchondral bones. The flat bones of the skull and face arise directly from mesenchyme and are called membranous bones.<sup>(29)</sup> It is useful to consider the enchondral skeleton as composed of two compartments, peripheral and central. The peripheral or appendicular skeleton is mostly composed of cortical bone while the central or axial skeleton mainly consist of trabeculae, contained within a cortical shell.

### **2.2.1. Cortical bone**

Cortical bone is composed of compact plate called lamellae, layer of bone, that are organized about central (Haversian) nutrient canals containing blood vessel and nerve.<sup>(30)</sup> Lamellae contact each other with lacunae, which contain branches of osteocyte and the bone extracellular fluid, serving as a nutrient canal to osteocytes. Lamellae, central canal, lacunae, osteocyte, and canaliculae are called osteon, a unit of bone. Osteocyte joins to each other and to the cells on the surface by filamentous prolongation of the cells that run within canaliculae. Cortical bone provides about three quarters of total skeletal mass or around 80% of total bone mass but only about

one-third of the total surface. It forms outer wall of all bones, but is almost abundant in the shaft of the long bones of the appendicular skeleton and some of the axial skeleton.

### **2.2.2 Trabecular bone**

Bone of the central or axial skeleton consist of a honey comb of vertical and horizontal bard, called trabeculae, 100 to 150  $\mu\text{m}$  in thickness that interconnect the interior surfaces of the cortical layer and surround the space of 500 to 1000  $\mu\text{m}$  diameter that contained haematopoietic tissue and fatty marrow.<sup>(30)</sup> Trabecular bone is found mainly in vertebral bodies, flat bones, and in the epiphyses of adult long bones. Trabecular bone provides the remaining one quarter of total skeletal mass, 25% of mineral, and about two-thirds of the total surface, which are important sites of bone remodeling, and serve to reduce skeletal weight without compromising the strength. Trabecular microstructure are relevant to fracture risk because the connectivity and space between the adjacent trabeculae are reduced with age which may cause bony porosity. It may result from perforation during resorption.

### **2.2.3 The differences of cortical and trabecular bone**

Cortical and trabecular bone differ in porosity and surface : volume ratio. By comparing the porosity of two types of bone, cortical bone is usually less than 10% while trabecular bone is more than 75% by volume but in aging skeleton, all degree of porosity may be founded. The ratio of surface to volume of bone is about four times higher in trabecular than in cortical bone, but because of the difference in porosity, the ratio of surface to volume of tissue is more nearly the same.<sup>(30)</sup>

Bone in different locations is subjected to different type of bone hence to differ biomechanical stresses and bone turnover. Radius and ulna comprise less than 20% of

cancellous bone except for their distal 2 cm which often taken to represent about 55 to 65% of cancellous.<sup>(31)</sup> Femoral neck comprise of 75% of cortical and 25% of trabecular bone whereas lumbar spine comprise of 35%of cortical and 65% of trabecular bone.<sup>(32)</sup> The proportion are measured by dual-energy absorptiometry. Furthermore cortical and trabecular bone differs in proximity to the bone marrow, blood supply, rapidity of turnover, timing and magnitude of loss with age, and influence on fracture risk.

The previous studies are noted that the rate of change of BMC (Bone mineral content) at different sites varies when compared with the rate of change of total body calcium.<sup>(33)</sup> This finding is not surprising, in view of the differential rate of loss of bone in various parts of the skeletons of osteoporotic patient. These differential rate are average in a total body calcium. Furthermore, patient who has low axial BMD are classified as normal at another site and vice versa. There is therefore a rationale for multiple sites of measurement including total body.

### **2.3 Bone acquisition in childhood and adolescence**

The acquisition of bone is paralleled with skeletal growth and increasing with age in healthy children. The accretion of bone mass increases rapidly during infancy, slowly through childhood and substantially increases again in puberty.<sup>(34)</sup> This is follow by a period of consolidation during which the mass of bone reaches its peak after which the bone then gradually loses its density.

Bone participates in three fundamental activities. **Modeling** refers to the process by which the characteristic shape of a bone is achieved. **Repair** is the regenerative

response to fracture. **Remodeling** is a stage of increase cavity to reduce weight but still have the same strength and continuous cycle of destruction and renewal that is carried out by individual and independent osteons, otherwise called bone remodeling units. In order to determine the acquisition of bone it is necessary to have a basic knowledge of normal bone modeling and remodeling and of the factors or mechanism involved in gain and loss of trabecular and cortical bone.<sup>(35)</sup>

### 2.3.1 Bone modeling

Bone modeling includes the combined processes associated with skeletal growth and maturation in childhood and adolescence, and with ensuring adaptive changes in bone shape.<sup>(35)</sup> Change in bone mass during the human life span fall into three periods; **bone growth**, **consolidation**, and **bone loss**. There is a progressive increase in bone volume until epiphyseal closure, both trabecular from endochondral ossification and cortical from the net periosteal apposition supplemented at different times and placed by net endosteal apposition.<sup>(31)</sup>

Bone growth occurs in epiphyseal plates by interstitial cartilage growth followed by calcification of the cartilage and eventual replacement by bone. In addition to the epiphyseal plates of long bones, smaller epiphyseal plates may exist in other bones for the elongation of certain bony projections such as the processes of the vertebrae. The epiphyseal plate is highly organized, and its cells are oriented parallel to the direction of growth. The chondrocytes nearest to the epiphysis are arranged randomly within the hyaline cartilage in the zone of resting cartilage and do not divide rapidly. Chondrocytes in the major portion of the epiphyseal plate, a region called the proliferating zone, proliferate rapidly and are organized into columns resembling stacks

of plates or coins; these columns paralleled the direction of growth, A maturation gradient exists in each column: cells near the epiphysis are younger and actively proliferate; whereas cells nearer to the diaphysis are older and are undergoing hypertrophy. As the matrix nearest to the diaphysis becomes mineralized with calcium carbonate, the hypertrophied chondrocytes die. Blood vessels grow into the area, bringing osteoblasts, which line up on the surface of the calcified cartilage trabeculae and deposit bone. Ossification and remodeling of the matrix then occurs. This process of proliferation, hypertrophy, calcification, cell death, ossification, and remodeling produces growth at the diaphyseal margin of the epiphyseal plate.

After cessation of bone growth, there is a period of consolidation during which the bone tissue density increase as bone turnover fall. At full pubertal development, the acquisition of bone mineral plateaus and final adult height are reached. Bone mass increase on consolidation by 5 to 10% and reach its peak about 35 to 40 years of age for cortical bone and probably earlier for trabecular bone.<sup>(36)</sup>

A recent longitudinal study using DEXA indicates that during pubertal maturation the accumulation rate in areal bone mineral density or content at both the lumbar spine and femoral neck levels increases four- to six fold over a 3- year period in females (from 11 to 14 years) and 4- year period in males.<sup>(37)</sup> The increase in BMD/BMC gain during corresponding pubertal period appears to be less marked in long bone diaphysis as observed on cross-sectional studies in the forearm or more recently in a longitudinal study at the level of the mid femoral shaft, a site where a twofold increase has been reported. There is a marked difference in magnitude in the increase in bone mass as compared with that in volumetric trabecular density. From

P5 the bony mass of lumbar vertebrae increases about tenfold more than the volumetric trabecular density. Thus, we observed that from stage P1 to P5, L2-4 BMC increase by about 175% and 160% in males and females, respectively.<sup>(11)</sup> In contrast, the corresponding mean increase in the volumetric trabecular density of the lumbar vertebrae as assessed by QCT were 20% and 15% in males and females, respectively.<sup>(10)</sup> This means that a large portion of the increment in bone mass during puberty is due to an increment in size associated with an increase in thickness of the cortical shell.

### 2.3.2 Peak bone mass

Although the precise timing and age of peak bone mass remains controversial and different among ethnic, several cross-sectional studies had demonstrated a plateau in bone accretion by late adolescence.<sup>(22,37,38)</sup> Peak bone mass can be defined as the amount of bony tissue present at the end of skeletal maturation and is an important determinant of osteoporotic fracture. Femoral neck and lumbar spine bone mass at this time is similar to adult levels, resulting in speculation that bone mass is at or near maximal levels by the end of the second decade. However, other studies have shown gains in bone mass in early adulthood. Healthy women followed longitudinally increased their bone mass up to age 29. The amount of bone mineral gained varied by skeletal site, with changes of 4.8% per decade in forearm bone mineral, 6.8% in vertebral bone density, and 12.5% for whole body.<sup>(39)</sup> By contrast, Barden and Mazess observed no increase in lumbar spine BMD among women ages 20 to 39 years studied longitudinally.<sup>(40)</sup> Even if bone mineral increases into early adulthood, it must be emphasized that the gains in the third decade are clearly small when compared with the

dramatic mineral accretion of adolescence so the factor which increase bone mass fully potential in childhood and adolescent to attain its peak is to be carefully considered.

### **2.3.3 Bone loss**

Bone is lost from every site amenable to measurement, especially from the endosteal surfaces in contact with the bone marrow, since the periosteal surface continues to gain bone slowly throughout life. In adult, the usual loss of bone density is about 1% per year.<sup>(41)</sup> In males, average cortical bone loss is about 0.3% of peak adult bone mass per year and trabecular bone loss slightly faster. In females, the average loss is about 1% of peak adult bone mass per year for both cortical and trabecular bone, with acceleration for about 5 years after menopause and a slower rate at both earlier and later time. The sex difference is greater for the femoral shaft than for the rib or the spine.

### **2.3.4 Bone Remodeling<sup>(42,43)</sup>**

Bone remodeling signifies the continuous renewal of old bone by osteoclast and osteoblast, predominantly take place at the Haversian system presented in cortical bone and the endosteal layer in trabecular bone, and is involved in changes of bone shape, the adjustment of the bone to stress, bone growth and repair.

Osteoblasts, a bone forming cell, synthesize the type I collagen, which is a major collagenase protein of bone and participate in the bone formation.

Osteoclasts are cells unique to bone and are always found in trabecular bone surface tightly associated with the calcified bone matrix. They are usually large, multinucleated cell which represent the functional cellular unit of bone tissue, and it is analogous to the nephron in the kidney. The bone resorption cell, osteoclast, is

responsible for demineralization of the bone matrix. The initial phase of osteoclastic bone resorption involves the attachment of osteoclast to the calcified bone surface.

As long bone increases in length and diameter, the sizes of the marrow cavity also increase. This proportional growth of lone bone makes it lighter with maintenance of bone strength and can bear more weight. Otherwise, the bone would consist of nearly solid bone matrix and would be very heavy when compared to a solid rod, a cylinder with the same height, weight, and composition but with greater diameter can support much more weight without bending. Therefore bone has a mechanical advantage as a cylinder than as a rod. The relative thickness of compact bone is maintained through bone absorption from endosteal layer by osteoclasts and laying down new bone from periosteal layer by osteoblasts.

#### **Characteristic feature of bone remodeling**

Normally, 90% of bone surfaces are at rest, covered by a thin layer of inactive lining cells. Remodeling is initiated by hormonal or physical signals that cause marrow-derived precursor cells to cluster on the bone surface where they fuse into multinucleated osteoclasts that in turn dig a cavity into the bone. The resorptive phase typically continues for 4-6 week. Osteoblasts then invade the area and form new bone for 3-4 month. This spatial and sequential coupling between resorption and formation to a large extent ensures skeletal integrity despite substantial variations in skeletal turnover. The outcome of this scenario is an osteon in cortical bone and a packet in trabecular bone.<sup>(35)</sup> Bone undergoes continuous remodeling in cycle of resorption, formation and quiescence.

If the remodeling cycles were completely efficient, bone would be neither lost nor gained. Each remodeling unit would be associated with complete replacement of the packet of bone that was initially lost. However, remodeling, like most biologic process, is not entirely efficient. The amount of bone replaced by formation is not always equal to the amount previously removed, so that a small bone deficit persists after each cycle. This inefficiency is called remodeling imbalance and is minuscule for any single normal bone remodeling event. Unless remodeling dynamics are perturbed, the resulting accumulation of bone deficits may be detected only after many years. The concept of remodeling imbalance is validated by the fact that osteomal thickness decreases with age. It is not certain whether remodeling imbalance always occurs or whether it develops with aging. Nonetheless, it carries the profound implication that age related bone loss is a normal, predictable phenomenon, beginning shortly after cessation of linear growth. Any stimulus that increases the overall rate of bone remodeling will increase the rate of bone loss.

#### **2.4 Factors affecting bone mineral density**

Many factors are supposed to influence bone mass accumulation which are independent of each other during growth. The list of these determinant includes : genetic, gender, dietary components (energy, protein, calcium), endocrine factor (sex steroids), and mechanical forces (physical activity, weight). More than half of the variance in adult bone mass can be attributed to genetic while nongenetic factors contribute to the remainder. <sup>(8,22)</sup>

Although genetic factors play a major role on bone mass and cannot be modified, they are theoretically immutable and could lead to a nihilistic approach that “nothing to be done”. Thus the public health focus on environmental and life style parameter which are calcium nutriture, activity level, body mass and endocrine status.

#### 2.4.1 Genetic/Ethnic determinants

Genetic factors are generally viewed as the most important determinant of bone mass. There are evidences that it contributes about 70-80% of the variance on bone mass and the remaining 20-30% is affected by environment.<sup>(8,36)</sup> The bone mineral status of their parents and children are highly correlated not only in mother and daughter,<sup>(44,45)</sup> father and son<sup>(46)</sup> but also parent and children.<sup>(46,47,48)</sup> Twin studies indicated that there were significant larger intra-pair difference between dizygotic than monozygotic twin.<sup>(49,50)</sup>

Recently, the studies indicated that allelic variation in the gene encoding the vitamin D receptor (VDR) may play a role in the regulation of bone remodeling and bone density.<sup>(51)</sup> The active hormonal form of vitamin D, 1,25-dihydroxyvitamin D, acts on the osteocalcin gene via the VDR and the vitamin D response element of osteocalcin gene.<sup>(51)</sup> Furthermore, polymorphism of vitamin D is found to be associated with bone mineral density. Using dizygotic twin as sibling pairs, allelic variation in the gene encoding the VDR was found to be linked to the trait of BMD in Caucasian adults. In contrast with Asian population, the studies reported that VDR gene polymorphism does not appear to be associated with BMD or bone turnover in postmenopausal women.<sup>(52)</sup> There was reported that B allele was found 16.6%, 60%, 74.9% in Asian, European, and African group respectively and bone mineral density in bb was highest

follow by Bb and BB respectively. The effect of allelic seem to be associated with bone mass in particular group.

The result presume that Bsm-1 vitamin D receptor gene polymorphism may have a physiologic role in calcium homeostasis by modulating intestinal calcium absorption. There was reported that subject with bb genotype had higher 24 hour urinary calcium excretion than those with Bb genotype although dietary calcium intake in subjects with bb and Bb genotype was not difference.<sup>(53)</sup> Bonjour et al also reported that the genetic mechanism will govern bone mass in childhood and adolescence through the effect of various physiological functions that in turn influence bone mass accumulation during growth and relative to calcium phosphorus metabolism.<sup>(22)</sup> Metabolism of calcium phosphorus has two main mechanisms during growth which are increment in plasma level  $1,25(\text{OH})_2\text{D}_3$  or calcitriol and the stimulation of renal tubular reabsorption of inorganic phosphate. The elevation in the production and plasma level of calcitriol enhance the capacity of the intestinal epithelium to absorb calcium and phosphorus. The increase in the tubular reabsorption of inorganic phosphate results in a rise in the extracellular concentration of inorganic phosphate. These two adaptive responses will promote optimal growth and mineralization. Moreover, the absorption of calcium are different among ethnicity. The U.S. RDA assumes that calcium absorption in Caucasian children and adolescence are less than 40% in contrast with studies in Chinese children who have the rate of absorption around 55-63%.<sup>(15)</sup> The difference in calcium absorption may be affected by VDR genotype.<sup>(53)</sup>

The study of the genetic mechanism may help identify those who are at greater risk of developing osteoporosis and may benefit from earlier prevention. Furthermore,

in children, understanding the genetic mechanism may help to promote peak bone mass in fully genetic potential and appropriate period of life. Further research needs to be done to clarify what is going on in genetic effect on bone mass.

Ethnic is one of factors that influences bone mass. Comparing between ethnic, black have higher areal bone density than white while white have higher areal bone density than Asian.<sup>(54)</sup> Schnitzler et al found that south African blacks have greater trabecular thickness compared with south African white especially in pubertal period<sup>(55)</sup> which are the same as result off Gilsanz and coworker who showed that volumetric trabecular density at spine was higher in black than white girls at puberty.<sup>(56)</sup> During childhood, there is little evidence about ethnic influences on bone mineral acquisition and most recent normative studies in children have been limited to Caucasian subjects.

#### **2.4.2 Age and gender**

Many studies reported that bone mass increase with advancing age and difference between gender. Although the influence of gender on bone mineral status remain dispute, most report but not entirely showed the correlation between gender at different age and skeletal site. In children, there are evidence that boys have greater radius and femoral neck BMD than girls<sup>(57)</sup> whereas prepubertal boys and girls have similar vertebral spine BMD.<sup>(58)</sup> In adolescents, girls have higher areal BMD than boys in lumbar spine<sup>(11,59,60,61,62)</sup> but volumetric BMD in lumbar spine is similar in boys and girls.<sup>(58)</sup>

The gender related difference may be explained by the onset of pubertal maturation which begins and completes earlier in girls. The greatest rate of accrual of bone occurred in girls 2 years before menarche, the period of greater change in level of

sex steroid hormones especially estradiol<sup>(24)</sup> There is still quite an increase in bone density up to the age of 20. In boys, the maximal increase usually occurs during the period of growth spurt (14.5 years) with slower accural of mineral up to 20 years of age.

Comparing to young adults, areal BMD is greater in male than female. It is obvious that this disparity results from bone mass gain in early life. Kroger et al found that size of measured bone areas (width of L<sub>2</sub>-L<sub>4</sub> and width of femoral neck) were closely correlated with growth parameter and the annual height gain was related to increase in BMD, BMC and bone size.<sup>(62)</sup>

### 2.4.3 Puberty

Bone accretion increase dramatically during adolescence, coinciding with the onset of puberty in both boys and girls. Since the pubertal growth spurt occurs simultaneously with the rise in sex steroids, which affect bone mineral acquisition. Some studies found that hypogonadal boys and girls show important deficit in cortical and trabecular bone mineral, and loss of endogenous androgen or estrogen during adult life accelerates the loss of bone,<sup>(62)</sup> especially estrogen which was claimed that its deficiency will decrease efficiency of intestinal and renal calcium handling, increase the calcium requirement, directly affects bone cell formation which result in acceleration of bone loss of early deficiency.<sup>(64)</sup> Thus we may say that estrogen plays a major role in bone mass regulation. If the sex steroid hormone is changed dramatically, bone mass will have high variations. The studies done by Ito and colleague found that the age at menarche may have a strong association with peak bone mass, as suggested by the positive correlation of early menarche with high BMD observed in their study.<sup>(13)</sup> It is

considered important to prevent risk factors that disturb the beginning of menstruation in adolescent girls.

#### **2.4.4 Dietary factor**

The optimization of skeletal growth has occurred because of the availability of high quality food containing sufficient amount of protein, energy and micronutrients especially calcium which is mineral deposit on bone. Deficient intake of these nutrients may disturb to process of bone growth and mineralization. Consequently, the poor calcified bone will suffer from rickets osteomalasia which the mineral content may be as low as 35%<sup>(29,30)</sup> or may be developed to osteoporosis when bone mineral density is two standard deviations below the young normal mean. By demineralization, bone loses most of its hardness but is very tough and flexible. On the other hand, if the organic components are extracted from a bone, the bone will lose its tensile strength and is as brittle as porcelain. Thus, it is apparent that the hardness of bone depends on its inorganic constituents, whereas its great toughness and resilience resides in its organic matrix, particularly in its abundant collagen fiber. The role of nutrition on bone depends on the aspect of bone function and the process of bone growth.

##### **2.4.4.1 Dietary calcium**

The most abundant of calcium compound (99%) is in bone and 1% of body's calcium is extra skeleton. The estimation of bone mineral content in young woman are approximately 2,200 gm which are 708 gm of calcium or 32.2% of total bone mineral content.<sup>(65)</sup> However the remaining calcium deposited on bone depends on balancing from calcium intake, calcium absorption and calcium excretion.

#### 2.4.4.1.1 Calcium balance

The control of body calcium requires a well balance interaction of different functional unit among calcium intake, absorption, and excretion. The normal level of serum calcium is required for daily growth especially bony structure, development, and tissue repair

The only source of calcium available to the body is the diet. Using vitamin D for trapping, majority of dietary calcium was absorbed in small bowel via both active and passive process. Active calcium absorption needs vitamin D and protein cytosol within the cell whereas the passive process depends on osmolarity flow.

Factors having impact on calcium absorption are age, sex, body calcium requirement, hormonal status (parathyroid hormone, growth hormone, estrogen), the composition and form of calcium in the diet. Adolescents have the absorptive power for dietary calcium of 40% as compared to adult which was only 20-40%. Lee et al stated that the amount of calcium absorbed is inversely proportion to the level of calcium presented in that diet.<sup>(15)</sup>

The body calcium was excreted daily via urine, feces and sweat in the amount 61, 35, and 10 mg.d<sup>-1</sup> respectively as compare to adolescent period which the excretion increase twice, being 112, 64, and 19 mg.d<sup>-1</sup> respectively.<sup>(69)</sup> Anyhow, bone is a major source of calcium replishment once the body needs it.

#### 2.4.4.1.2 Calcium retention

The study of calcium balance is important to estimate calcium retention on bone which more or less are different at any stage of life cycle. Considerable quantities of calcium deposits in the body between birth and maturity. The amount of calcium expressed relative to body weight increases from approximately 8 g Ca/kg at birth to 19 g Ca/kg in a 70 kg man.<sup>(28)</sup>

The rate of calcium accretion over 20 years is approximately 35 g/y or 97 mg.d<sup>-1</sup>.<sup>(24)</sup> The recent studies suggest that the rates of calcium accretion are different at any stage of development or sex. During adolescence, the rate of bone mineral retention increases rapidly in boy from 115 g/y to 320 g/y at the time of growth spurt (14.5 year of age). By comparing between sex, boy has greater bone mineral increment rate than girl from childhood through adolescent.<sup>(24)</sup> Studies of calcium balance in girls found that the positive calcium balance generally occurs at the end of late adolescent only if calcium intake approach more than 1000 mg/day.<sup>(66)</sup> Nordin suggest that recommendation in children is made for an RDA of 1,200 mg during childhood and 1,500 mg during adolescence to provide optimal calcium retention.<sup>(67)</sup> If the requirement is not met, the lower bone mass may increase incidence of radius bone fracture during adolescence or osteoporosis in later of life.<sup>(68)</sup>

#### 2.4.4.1.3 Calcium requirement

The recommended amount of calcium intake was about 800 mg in children and 1200 mg in adolescents.<sup>(69)</sup> The estimation of calcium requirement is the amount needed to compensate for endogenous obligatory losses and to allow for adequate calcium retention during the period of bone development. This calculation is based on

40-60% of true calcium absorption which more or less varies among ethnicity. There are evidences that Asian children who are accustomed to low calcium intake is able to absorb more calcium than the western ones,<sup>(15)</sup> while calcium excretion is less to maintain calcium balance. In recent study found that the positive calcium balance generally occur at the end of late adolescent only if calcium intake approach more than 1,000 mg/day.<sup>(66)</sup>

#### **2.4.4.1.4 The relationship between calcium intake and bone mineral status**

The relationship between calcium intake and bone mineral status remains controversial. Some cross-sectional studies show a significant correlation between calcium intake and bone status<sup>(70,71,72,73)</sup> but the other could not find the relationship.<sup>(38,57,59,74,75,76)</sup> These difference effect of dietary calcium intake on bone mass may be explained by many reasons. First, the sample sizes are inadequate to detect a small contribution of calcium to bone mass. Since there are marked effect of puberty and body weight, about 70-80% of variability, the remaining is dietary calcium intake which contributes approximately 20% of variance to bone mass.<sup>(38,72)</sup> The relatively small sample size of subject may be insensitive to an effect of this magnitude. Second, in growth spurt, the trend of calcium intake is diminished by comparing with small children. While the high increment rate of bone gain appears, the relationship of calcium and bone mass will be occulted. Third, the difference in method of dietary assessment which was used in variety. Calcium nutriture which affect the bone status must be an habitual calcium intake because calcium itself is a nutrient which has high variation both within and between subjects. If the researchers would like to assess the

correlation of calcium nutriture they must use the accurate method suitable for research question. Lastly, calcium intake was not associated with bone parameter because of the high level of calcium intake in children.<sup>(57,74,76)</sup> These evidence was supported by Matkovic and Heaney that calcium is considered a threshold nutrient which means that calcium intake exceeding threshold level does not further contribute to bone mineralization.<sup>(67)</sup> However, dietary calcium appeared to be a determinant of bone mineral density, especially before puberty in children who consumed calcium less than 1000 mg/day.<sup>(73)</sup>

The intervention studies of childhood and adolescent growth also support the influence of calcium intake on bone mass. Johnston et al<sup>(8)</sup> found that 45 identical twin pairs receiving daily calcium supplement of 700 mg had greater increase of bone mineral density than their placebo twins, which is the same as the result of Slemenda et al.<sup>(78)</sup> The evidence in Asian studies done by Lee and colleague also support that calcium supplement (300 mg Ca/day) in children age 7 years old who had low calcium intake (280 mg/day) had moderate effect on bone mineral accretion<sup>(15)</sup> and the effect of calcium supplement (800 mg Ca/day) on bone mass in 7 year old children, whose calcium intake was 567 mg /day, was found at the spine and radius but no effects on femoral neck.<sup>(79)</sup> Gains in bone mass with these studies were significant only in girls who remained prepubertal. The benefit of calcium supplementation disappeared after treatment was withdrawn.<sup>(78,79)</sup> These findings support that diet calcium during childhood should be adequate especially at the time before puberty to achieve optimal skeletal accretion which allow individuals to reach their full genetic potential for

acquiring skeletal mass.<sup>(80)</sup> If they cannot change any habit, the determination of supplementation may replace to enhance peak bone mass effectively.

#### **2.4.4.2 Phosphorus**

Phosphorus is the second major mineral in human body. Eighty five percent is in the skeleton, the remainder is distributed between tissue and membrane components of skeletal muscle, skin, nervous tissue and other organs. Most of the phosphorus in soft tissue and cell membrane is in the form of organic ester, almost all the phosphorus in bone is contained in the mineral phase as inorganic orthophosphate and small amount of inorganic phosphate. Phosphate function play a fundamental role in human body. Concerning the development and maturation of bone, phosphate plays a role in the formation of the apatic structure, affect bone resorption, mineralization, and collagen synthesis and thus play an intregal role in calcium homeostasis.

Phosphate is as important as calcium for bone health because it contributes to about 50% of bone mineral weight. Calcium and phosphate levels should be present in adequate quantities in the diet to ensure proper bone mass development. Dietary calcium and phosphorus intake are highly correlated because of a common origin in dairy products. Recommended dietary allowance (RDA) for phosphorus level are arbitrarily set to be the same as calcium RDA; 800-1200 mg in children and adolescents.

According to the 1989 RDA, the desired Ca:P ratio of the United States diet is 1:1, but the ratio of the actual food consumption pattern is lower. The report of national nutrition finding survey show high phosphorus, low calcium intake to be characteristics of teenagers and young adult.<sup>(81)</sup> It is possible because of the self selected particular food group (meat, soft drink and commercial food) provided

phosphorus without calcium. Excessive phosphate intake has possible detrimental effect on bone mass because diets high in phosphates and low calcium have been shown to increase serum parathyroid hormone (PTH) level.<sup>(82)</sup> PTH acts primarily at the kidney by enhancing the tubular reabsorption of calcium and stimulating the formation of calcitriol, but also at the skeleton by mobilizing or storing calcium. Calcitriol stimulates intestinal calcium absorption, affects bone turnover, and down regulates its own formation and the synthesis of PTH.<sup>(9)</sup> The effect of a moderately low calcium, high phosphorus diet is progressive secondary hyperparathyroidism and a modest reduction in trabecular bone mass as found in animal study. In population study, the role of the high phosphorus, low calcium composition on bone health have been confounded by the effects of protein intake.<sup>(81)</sup>

#### **2.4.4.3 Protein and energy**

Animal protein thought to be beneficial for bone is now regarded as a positive risk factor because of its tendency to increase urinary calcium excretion. This results from the combined effects of the acid load and the sulphate radicals on tubular reabsorption of calcium.<sup>(83, 84)</sup>

#### **2.4.4.4 Sodium**

Dietary sodium is potentially more important risk factor. The positive effect of salt ingestion on calcium excretion has been reported for many years and is the result of competition between sodium and calcium for proximal tubular reabsorption.<sup>(85)</sup> When absorbed calcium exceeds requirement, the surplus is shed in the urine and urine calcium becomes a function of absorbed calcium via the filtered load; calcium is pushed out. When absorbed calcium falls short of requirement, calcium in the urine is pulled

in; the rate of excretion is largely determined by tubular reabsorption, which is highly sodium-dependent. Urine calcium is therefore a function of dietary sodium in the fasting state and in the 24-hour urine of subjects whose calcium intake and/or calcium absorption is low, but not in the 24-hour urine if the calcium intake and/or absorption is high.

#### **2.4.5 Physical activity**

Physical activity is one of the determinants of bone strength by Wolff's law which states that bone will adapt to mechanical loading applied to it. This adaptation included increase in density when mechanical loading are increased and no further changes will occur unless the stress level is modified. Mechanical force may be applied to the skeleton by gravitational or weight bearing and non weight-bearing muscle tension. Reduction of mechanical force (weightlessness, bed rest) will alter calcium metabolism by increasing urinary calcium and stimulate bone resorption<sup>(86)</sup> which in turn affect on bone mass. Apart from force, blood supply also play a role to relate with bone strength because bone adjacent to muscle will be more dense than the distance one.<sup>(87)</sup>

Physical activity probably contribute to increase bone density especially in childhood and youth.<sup>(88,89)</sup> The effect of physical activity on skeletal mass depends upon type, intensity, duration and frequency of either mechanical force or exercise. There are widespread acceptance that weight bearing activity (such as running, basketball, football, base ball, tennis etc.) or weight resistance activity (weight lifting etc.) have significant greater impact on bone mass than sedentary control in cross sectional studies,<sup>(76,90)</sup> prospective studies<sup>(88,91)</sup> and interventional studies.<sup>(87,92)</sup> Moderate to high level of exercise contribute to greater bone mass than control

group.<sup>(26,72,90)</sup> However, evidence justified optimism for the concept that regular physical exercise benefits the skeleton.<sup>(93)</sup> The weekly duration of activity should be greater than 7 hours which will have positive effect on bone mass gain.<sup>(25)</sup>

The response of bone to mechanical loading is site specific and not generalized throughout skeleton. Areal BMD of gymnastic athlete is greater at arms and legs in weight bearing site but not at skull.<sup>(94)</sup>

Since the most rapid rate of bone growth and development occur prior to puberty, the appropriate time to modified or promote optimal bone growth are prepuberty.<sup>(26,95,96)</sup> Furthermore, at this time, there is no sex dependent effect in primary amenorrhea or delay puberty of strenuous exercise.

#### **2.4.6 Body Mass**

Body mass has been a significant predictor of bone mass especially in young healthy children. Body mass can be expressed as weight, height or body mass index. The different in body mass can infer to relate with bone size than bone mass in term of bone mineral. Bone size affect mostly on BMC, intermediately in BMD<sup>(59)</sup> and without significance to volumetric bone density.<sup>(97)</sup>

The relationship between body mass and bone mineral status may not be solely an artifact of the terms used to estimate bone mass. Increased body mass increase the mechanical load on the skeleton, which may directly contribute to bone mineral accretion. Furthermore, body mass reflects overall nutritional status, low body mass appears to be an important risk factor for osteopenia in children and young adults.

Factors that influence bone density also interact with each other. Body mass relate with puberty; it is difficult to separate the contributions of body mass and sex

hormone to bone mineral gains. The time of peak growth velocity is the same timing as the highest period of sex hormone. The effect of sex hormone itself on development of bone mass or the indirect effect to body mass before bone mass are still controversial. Katzman et al propose that increased concentrations of sex steroid either by direct action on bone or indirectly through stimulation of other mediators of bone growth has an impact on bone mass.<sup>(38)</sup> However, pubertal stage has been shown to predict bone mineral status independently of body mass, supporting an independent effect of sex steroid on bone mineral accretion during adolescence.

## 2.5 Bone status measurement

All absorptiometric techniques rely on the attenuation of energy that occurs as the beam of penetrating x-ray photons scan across a region of interest or the whole body. Bone edges are detected by using software algorithms based on the amount or change of attenuation over a certain scan distance. The cumulative attenuation between bone edges is converted into mass of mineral within the bone envelope. The results are expressed as bone mineral content (BMC, g) and bone mineral density (BMD,  $\text{g}\cdot\text{cm}^{-2}$ ) which is derived from BMC divided by the scan area of bone.

It is slightly different in reflecting bone status by using both of BMC and BMD. Using absolute amount of BMC per se is not sufficient for evaluating the degree of calcification of an individual because its degree varies markedly on the basis of body size, sex, age, and body habitus.<sup>(98)</sup>

The expression of bone mineral data as BMD does provide a degree of standardization for differences in bone size between individuals and facilitate the

comparison of an individual with a reference population.<sup>(99,100)</sup> BMD has been shown to be a useful predictor of future risk and to provide good discrimination between patient with osteoporosis and normal individual. It also has the advantage that measurement error, due to slight movement or differences in bone edge detection, tend to affect both BMC and BA (BW) in the same direction. Consequently, the reproducibility of BMD tends to be better than that of BMC, which can be advantage especially in longitudinal studies within individual.

Bone mineral density (BMD) are often used as true bone density, areal bone density and whole bone density. True bone density is a function of content (BMC) per volume of bone, that is volumetric BMD (vBMD, gram per cm<sup>-3</sup>). Areal bone density (aBMD) is an amount of bone mineral per area (BMC per cm<sup>-2</sup>). Whole bone density or apparent bone density obtain an entire bone, including mineralization and unmineralization.

The increment of bone mineral density did not find changing in number per unit area or unit volume but depend on thickness of either cortical or trabecular bone. The cortical density is determined by the increase in thickness of cortice or cortice with fewer or smaller Harversian canal. Cortical thickness is the integrity of periosteal and endosteal growth which is responsible for 77%, 23% in white men and 65%, 35% in white women respectively.<sup>(54)</sup> These proportion are the same in children from Central and North America but not reported in Black and Asian children. Trabecular thickness is important in term of the rate of bone loss or remodeling process. Comparing the number and thickness of trabecular bone between two women with identical volumetric bone density, if ones has double the number of trabeculae of half the thickness, the

trabecular surface area will be twice that of the women with half the number of trabeculae of double the thickness.

### *Interpretation of bone status*

Bone growth in children will lead to a much greater change in bone volume than in bone area and may lead to an inappropriate substitution of aBMD for vBMD. So it is necessary to take into account because it can influence in interpretation of the bone status. Carter and colleague, comparing two different bone size and thickness, have shown that a smaller bone has the same areal bone mineral density as the bigger one but not in volumetric bone density.<sup>(101)</sup>

## **2.6 Osteoporosis**

Osteoporosis is a disorder of generalized skeletal fragility and can cause osteoporotic fracture, an inappropriate low bone strength. There is no absolute fracture threshold; fracture risk is already increased in the lower part of the young normal range. It may be reduction in bone density which is therefore attributed to increasing fracture risk. A bone density more than two standard deviations below the young normal mean at the same site and in the same sex is osteoporotic, bone densities between one and two standard deviations below the young mean as “mild osteoporosis”; those between two and three standard deviations below as “definite osteoporosis”; and those more than three standard deviations below as “severe osteoporosis”. The risk sites of osteoporotic fracture are common in radius, femur, and lumbar spine. These fractures are found in later of life when the rate of bone loss occurs rapidly. In children, transient osteoporosis of childhood is often referred to as

idiopathic juvenile osteoporosis, usually becomes event just before puberty. There are evidences to support that transient osteoporosis of childhood results from reduced osteoblastic activity that is unable to keep pace with a normal resorption rate. The characteristics of transient osteoporosis may present in terms of increment of hydroxyproline excretion and negative calcium balance. The negative calcium balance could be related to decrease serum 1,25 dehydroxy vitaminD levels, which were observed in one patient with transient osteoporosis of childhood, may be related to decreasing calcium absorption. A positive calcium balance, however, may be seen in recovering or healed patient. The incidence of fracture in children occur at the distal end of radius which increase markedly at the time of peak velocity of growth.<sup>(68)</sup>

## CHAPTER III

### MATERIAL AND METHOD



#### 3.1 Selection of study subjects

Two hundred seventy six children, aged 9-12 years, living in Bangkok metropolitan area participated in this study. All participants were healthy, as evaluated by physical examination which was performed by pediatrician, which were recruited from Payathai and Samsenvithayalai School by using the following criteria:

1. Having normal growth and development based on standard weight and height for age of U.S. NCHS (National Center of Health Statistics), i.e. within  $\pm 2$  SD of the reference values.
2. No history of chronic illness or bone disease, no fracture and no history of exposure to any medication such as steroid or hormone.
3. Willing to participate in the study after receiving a written informed consent from parent or legal guardians.

This project has been approved by the ethical committee of the Faculty of Medicine, Ramathibodi hospital, Mahidol University.

### **3.2 Overview of study design**

In this study, we used an approach of cross-sectional study design to determine calcium intake and bone status of healthy Thai children aged 9-12 years.

Information on physiological factor such as age, sex, pubertal stage, weight and height as well as environmental factor which included usual and current calcium intake and related nutrients and weight bearing activity of each study subjects was also determined at the time of bone measurement.

### **3.3 Study site**

The study was carried out at the pediatric nutrition unit, Department of Pediatrics, Faculty of Medicine Ramathibodi hospital, Mahidol University.

### **3.4 Methods of data collection**

#### **3.4.1 Dietary assessment**

Usual and current dietary calcium intakes of each subject were assessed by using a modified semiquantitative food frequency questionnaire designed to estimate calcium intake from Thai diet during the past 6 months and a 3-day food record.

**3.4.1.1 3-day food record:** Daily nutrient intake was estimated from 3-day food record. The respondents were asked to record all food items and amount they consumed during 3 consecutive days before coming to the research unit. The time of each meal, list and amount of foods and beverages consumed including brand names, methods of food preparation and the amount of condiments or seasoning added to the

dish were recorded. The respondents were also asked to record the amount of whole dish consumed in order to recheck the accuracy of food record. Information was also obtained on whether the meal was eaten at home or at a commercial food establishment and whether the meal was eaten alone or with other person.

At the end of the 3-day food recording period, the students were asked to return the record form for verification of completeness and accuracy by the research team at Pediatric Nutrition unit, Faculty of Medicine, Ramathibodi hospital. They were asked to provide additional information about any unclear food item both in composition and quantity. Pictorial food model, measuring cups and spoons and a face-to-face dietary interview were used to increase the completeness and accuracy of estimation of size and amount of each food item. The amount and type of condiments and seasonings used during cooking was estimated by using the standard recipes from the well-accepted recipes of Thai food.<sup>(102,103)</sup> Emphasis was also placed to each subjects on the need to record all food items consumed at the time of each meal, and not from memory at the end of the day.

#### **3.4.1.2 The semi-quantitative Food-Frequency Questionnaire**

The Semi-quantitative food-frequency questionnaire was developed to estimate calcium intake from Thai diet during the past 6 months. This questionnaire consisted of three components: a list of food, a set of frequency of use response categories and a portion size. Foods to be included to the questionnaire were selected in 3 ways. Initially, we utilized the information of food items from 24-hour dietary recall collected from student grade 4-6 at Watmaipiren school, Bangkokyai, to identify food commonly consumed by school age children. Next, to ensure a full coverage of food

items we further identified potentially important sources of dietary calcium from food composition database of Thai diet.<sup>(104,105)</sup> Finally, some foods, such as snack foods, Thai style dessert, bakery products, beverage and food supplements were included. The questionnaire ultimately included 15 food groups, 44 food subgroups and totally 250 food items including beverages.

The questionnaire was pre-tested to check face validity and content validity with students at Mahidolvidhayanusorn school.

Administration of the semi-quantitative food frequency questionnaire subjects were given detailed instruction on how to complete the questionnaire before the date of appointment.

For each food items, firstly the respondents were asked to indicate on the average food items they had consumed during past 6 months. Secondly, for each food item that they have eaten, they were asked to indicate the average frequency of intake. These were nine possible responses ranging from never to four or more times a day. At the end of each food group was an open-ended section in which the subjects were asked to note any other foods eaten at least once a month and to describe the frequency of consumption and typical portion of those foods. Thirdly, the respondent were asked indicate, on the average, the amount of consumption of each food items.

#### **3.4.2 Bone mineral measurement<sup>(106)</sup>**

The bone mineral measurement was performed for total body and three skeletal sites namely; midshaft radius, lumbar spine, femoral neck by using dual-energy x-ray absorptiometry (DEXA, Lunar Corporation, Medison, U.S.A.). This absorptiometry technique was an x-ray tube operating constant potential kiloelectron voltage and 10

mA coupled with a samarium filter (K-edge 46.8 KeV) which effectively separate the spectrum into two energy peaks. The x-ray detector consists of two sodium iodide scintillation detectors that measure the transmitted x-ray beam intensities at two energies, high energy (76 keV) and low energy (38 keV). High energy level counts bone but low energy level counts soft tissue by air count mode 430,000 times/second, and 710,000 times/second respectively.

#### **3.4.2.1 Site of measurement**

The following measurement were carried out in each subjects:

1. Total body bone mineral content (TBBMC), total body bone mineral density (TBBMD) and total body calcium (TBC)
2. Midshaft radius bone mineral content (midshaft radius BMC), midshaft radius areal bone mineral density (midshaft radius aBMD)
3. Lumbar spine bone mineral content (L2-4 BMC), lumbar spine areal bone mineral density (L2-4 aBMD), lumbar spine volumetric bone mineral density (L2-4 vBMD)
4. Femoral neck bone mineral content (FNBMC), femoral neck areal bone mineral density (FN aBMD), femoral neck volumetric bone mineral density (FN vBMD)

#### **3.4.2.2 Operation of bone mineral measurement<sup>(106)</sup>**

DEXA is automatically controlled by computer. The technician operated the machine through computer, arranged the position of subject and indicated the centering point and region of interest (ROI) for each measurement site.

### Step in bone mass measurement

1. Subjects were asked to change their clothes to the hospital gown, and remove all metal object, e.g. ring, earring, necklace, etc.

### 2. The positioning of the subjects for bone mass measurement

#### 2.1 Radius

2.1.1 Subject sat down on the chair beside the scan table or measuring platform with the volar side of his/her slightly clenched hand in non dominant arm and upper arm resting on the table and then located forearm on the forearm support which had the red line in the middle, and fastened forearm with black tape.

2.1.2 The squab of a seat was located against the red line

2.1.3 Measure the distance between the styloid process and olecranon process (key in computer program) and measure the width of forearm in the largest part and plus 2 centimeter (key in computer program).

2.1.4 Set the centering point which was 1 cm. above the palpated proximal wrist joint (styloid process) and the end point was approximate a middle of distance between styloid process and olecranon process. The scan speed was fast, 150  $\mu$ A, sample size 0.6×1.2 mm. Total scan time was approximately 2-3 minutes (depending on length and width of forearm).

#### 2.2 A-P spine

2.2.1 Subject laid supine on the scanning table, lifted his/her legs onto the support block which helped separate the subject vertebrae and straighten the

curve in the subject's lower back. Position the block so the subject's thighs were at a 60-90 degree angle to the table top.

2.2.2 Measure centering point which was 1.5 inches below navel.

This was a margin of lumbar spine to cover the interesting part (L<sub>2</sub> - L<sub>4</sub>).

2.2.3 Start scanning from center point to the twelfth thoracic spine (at the middle of the body spine) with the medium speed, 750  $\mu$ A, sample size 1.2×1.2 mm. Total scan time was approximately 6 minutes.

### 2.3 Femoral neck

2.3.1 Subjects laid supine on the scanning table in the supine position

2.3.2 Strap the left foot into the triangular foot brace. Fasten the foot of the leg which was going to image to the angled side of the brace (25 degree) and then rotate the entire leg to the angle of the brace. Do not just rotate the foot. The lower leg was supported with the sponge to relax. Fasten the other foot to the straight, vertical side of the brace.

The subject's toes should point upward, not towards the end of the table. It is important to confirm that patient's feet are firmly attached to the foot brace. The subject's femoral shaft must be paralleled to the center line on the table pad to ensure sufficient space between the femoral shaft and ischium for accurate femur analysis.

2.3.3 Measure centering point which was a lower border of symphysis pubis or 8 inches below iliac crest.

2.3.4 start scanning from centering point to 3 centimeters above greater trochanter with medium speed, 750  $\mu$ A, sample size 1.2×1.2 mm. Total scan time was approximately 3 minutes.

## 2.4 Total body

2.4.1 Lay down on the scanning table. All of the body was within the inner blue frame by head of subject attached the blue line.

2.4.2 Move detector to the skull and start scanning with fast speed, 150  $\mu\text{A}$ , sample size 4.8 $\times$ 9.6 mm. Total scan time was approximately 10-12 minutes.

3. Move detector over scanning part and press start button. The x-ray tube which was under the scanning table will release the photon.

4. Analyze bone mineral density and body composition data from the programmed computer.

### 3.4.2.3 Analysis of result of DEXA

The detector will absorb ionizing radiation by bone. The amount of radiation attenuation is directly related to the amount of bone present. Post acquisition processing of the data allow definition of region of interest and calculation of the projected area of these regions. Bone mineral density is calculated by dividing the total bone mineral in grams (BMC) by the projected area of the specified region. Then soft tissue will absorb radiation attenuation which produce by low energy and calculate lean mass, fat mass etc.

### 3.4.2.4 Volumetric BMD calculation

DEXA indicates the parameter of bone mass as BMC and areal BMD. Since this method does not correct for anterior-posterior depth, the BMD obtained from DEXA is greatly influenced by bone size. To minimize this error, bone volume was calculated on this two dimension DEXA measurement at lumbar spine and femoral neck. The height and width of lumbar spine and the left femoral neck was provided by regional

analysis of total body software. Both femoral neck and vertebral bodies were hypothesized to have a cylinder shape and the volume of bone was calculated<sup>(97)</sup> as follow:

$$\text{volumetric BMD} = \text{BMC} / \text{volume}$$

**Femoral neck; volume**

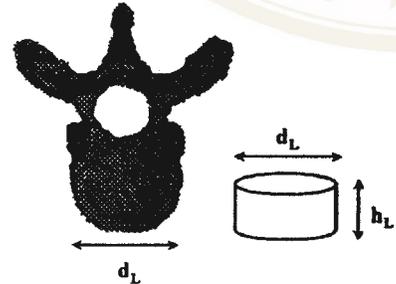
$$= \pi \times r^2 \times h$$

$$= \pi \times (\text{width}/2)^2 \times h$$

$$= \pi \times (d_n/2)^2 \times h$$


**Lumbar spine; volume**

$$= \pi \times r^2 \times L_{2-4} \text{ (length)}$$

$$= \pi \times (d_v/2)^2 \times L_{2-4} \text{ (length)}$$


### ***Quality assurance (Q.A)***

Quality assurance was performed in the morning daily. Q.A of bone densitometer composed of regional self test and quality control of its manufacture. Bone densitometer would be functioned if Q.A was shown “pass” only. Furthermore, quality control of phantom spine was used to standardize BMD value for error protection.

### **3.4.3 Physical examination and sexual maturation assessment**

Physical examination and determination of sexual maturation was performed by a pediatrician. The classification of pubertal stage for genital stages in males and breast development in females was determined according to the criteria of Tanner. In this study, the subjects were classified as pre-puberty (Tanner stage 1) early puberty (Tanner stage 2 and 3) and late puberty (Tanner stage 4 and 5) (Appendix A).

### **3.4.4 Anthropometric assessment**

Body weight of children was determined using a Seca electronic scale while the subjects were wearing light school uniform without shoes and recorded the body weight to the nearest 0.01 kg. The children’s height was measured by using a Seca stadiometer which was mounted on a Plexiglas frame in a standing position without shoes and recorded their height to the nearest 0.10 cm. Weight and height were calculated as Z-score of NCHS reference according to age and sex.

### **3.4.5 Physical activity**

Physical activity was measured by using the self-administered questionnaire which asked about habitual physical activity including during physical education classes, organized sports and recreational activity within the past 6 months. These activities were weight bearing ones (involving gravitational force on bone) which were indicated

to have more beneficial effect on bone mass than the activities which were supported by seat or by water (such as bicycling and swimming). The children were asked to indicate the average number of hours per week for each activity. The summation of total activity was calculated to determine the relationship on bone mass. A list of weight bearing activities in this study were presented in Appendix D.

### 3.5 Data analysis

#### 3.5.1 Dietary data analysis

Daily consumption of macro nutrient, total energy, calcium, phosphorus and sodium of each subject was derived from the 3-day food record whereas usual calcium intake was derived from sFFQ.

##### *Calculation of nutrient intake and food groups*

The weight in gram of each food item was converted from household measurement by using the food conversion manual<sup>(107)</sup> and direct weighing by the research team. For 3-day food record, all items were coded by food items and amounts. The computation of nutrient intake was made by using the computerized food composition analysis package INMUCAL of the Institute of Nutrition, Mahidol University.<sup>(108)</sup> For the sFFQ, calcium intake were computed by multiplying the amount of calcium per 100 gram of each food item with the quantity of each food item (in gram) and number of time consumed per month. Then the calcium intake was converted into milligram per day.

### 3.5.2 The validation study

In this study, the semiquantitative food frequency questionnaire (sFFQ) was validated to test whether or not the result obtained from sFFQ could reflect the true intake of the study subjects. Dietary assessment technique ranged from record with weights of food to recall by using both self-reported and in the form of questionnaire. Method of use in dietary assessment depended upon a variety of factors such as, the purpose of the study, the difference in nutrient intake estimation, validity or accuracy of dietary assessment technique, systemic measurement error or bias and etc. Validity is important in the design of nutritional assessment system because it describes the adequacy with which and measurement or index reflect the nutritional parameter of interest. If we would like to assess the long term nutritional status of an individual, the dietary measurement should provide a valid reflection of the true “usual” nutrient intake rather than the intake over a short period of time. The design of a valid and reliable nutritional assessment system must include consideration of the validity of the selected method especially the new approach technique which had never been used before.

The food frequency questionnaire is a practical tool for the measurement of usual food consumption pattern in a population because it is inexpensive and fairly easy to administer. The food frequency questionnaire was initially designed to define eating pattern of individual. Its principle advantage was in ranking individual according to their level of nutrient intake as compared within the group, not in quantifying individual intake.<sup>(109)</sup> Recent studies tried to introduce the semi-quantitative food frequency questionnaire to measure both of qualitative and quantitative data aiming to describe the possible relation between diet and disease. Since the semi-quantitative food

frequency questionnaire was a new method, it must be validated against more detailed and accurate method of dietary assessment.

The validation of dietary assessment technique was complicated by lacking of an ideal standard since the true value of nutrient intake remain unknown. Instead indirect methods were used, and a new method had to be evaluated by comparison with an established technique. If the new method agree with the old one, the latter may be replaced.<sup>(110)</sup>

Three-day food records were typically considered to be the reference standard for determination of difference between groups of individuals in nutrition and epidemiological researches for overall food and nutrient intake. Other methods were acceptable for each of these purpose if they were shown to be as valid and precise as recording methods.

In this study, we would like to determine the ability of sFFQ to assess usual calcium intake of the study subjects with an acceptable limit of inaccuracy. The result obtained from sFFQ and 3-day food record over the same period were compared to determine the consistency of the results of these two techniques to indicate group mean intake, individual intake and in categorizing the subjects according of the level or amount of their intake.

Twenty-two children (13 boys, 9 girls) aged 11-14 years from Samsenvitayalai school agreed to participate in this validation study. They were requested to complete 3-day food record for each month which include 2 weekdays and one weekend for 6 months. By the end of 6 months, the subjects were asked to complete the sFFQs which

was designed to obtain the information of food intake both in terms of frequency of consumption and the amount eaten. The period covered by the sFFQ was 6 months.

All records were coded for analysis and calcium intakes were computed using an updated version of INMUCAL of food composition database for calcium project. The value of calcium intake from sFFQ were computed by converting the quantity of consumed foods to weight of food, expressed in gram. Calcium intake was then evaluated by multiplying consumption frequency (times per month), weight of food (in gram) and the amount of calcium content of each food item (mg/100g of edible portion) based on Thai food composition database completed by the researcher at the Institute of Nutrition, Mahidol University. Calcium intake (g/month) in each food items was aggregated into total calcium intake and was then converted to daily total calcium intake.

### **Data analysis**

Mean, standard deviations and ranges of calcium intake were calculated from both the diet record and the sFFQ. To test the consistency of the two methods of dietary assessment estimating group mean calcium intake both as mean absolute intake or crude data and as nutrient density, the t-test for paired samples was used to test for differences between methods for males and females separately and between groups as well. Consistency in categorizing individual children at the extreme quartiles of intake was evaluated using contingency. The percentage correctly classified in the extreme quartiles when they were categorized by the food record method in the highest (lowest) quartile of the distribution for calcium and were classified as being in the highest (lowest) quartile according to sFFQ, and the overall percentage correctly classified

within one quartile when they were categorized by the food record method in the highest (lowest) quartile of the distribution for calcium and were classified as being in either the highest (lowest) quartile or the highest (lowest) two quartiles according to the sFFQ were calculated. Due to chance alone, 62.5% of subjects would be expected to be correctly classified to within one quartile.

### Results

Mean age of the study subjects was  $12.8 \pm 0.79$  years and  $12.3 \pm 0.64$  years for boys and girls, respectively. Mean calcium intakes were not different between males and females in both sFFQ ( $695.7 \pm 323.8$  vs  $599.0 \pm 359.4$ ,  $p = 0.143$ ) and FR ( $586.3 \pm 249.1$  vs  $469.4 \pm 239.3$ ,  $p = 0.301$ ).

Table 3.1 demonstrate mean, standard deviation, minimum and maximum value. Calcium intake from sFFQ tended to be higher than FR in males, females and total subjects. The percentage of difference in absolute value were 18.7% (mean difference, 109.4) in males, 27.6% (mean difference, 129.6) in females and 23.5% (mean difference, 121.4) in total subjects. For nutrient density, the percentage of difference were 15.3% (mean difference, 58.5) in males, 22.7% (mean difference, 91.4) in females and 19.8% (mean difference, 78.0) in total subjects.

**Table 3.1** Estimates of group mean dietary calcium intake of children subjects, obtained from 6 three-day food records and semi-quantitative sFFQ

Dietary calcium intake		Absolute intake		Nutrient density	
		FR	sFFQ	FR	sFFQ
Males	mean	586.3	695.7	323.0	381.5
	SD.	249.1	323.8	123.7	149.5
Females	mean	469.4	599.0	312.1	403.5
	SD.	239.3	359.4	143.2	224.9
Total	mean	517.2	638.6	316.5	394.5
	SD.	244.6	340.8	132.6	193.7

**Table 3.2** Cross-classification of dietary calcium intake of children subjects from 3-day food record and sFFQ

Dietary calcium intake	Sex	FR absolute value				
		Correctly			total	Within-one quartile
		Highest quartile	Quartile 2-3	Lowest quartile		
sFFQ	M	3.33	22.22	-	44.44	100
absolute value	F	15.38	7.69	15.38	55.55	69.2
	Total	22.72	13.64	9.10	45.45	90.9
		FR nutrient density				
sFFQ	M	11.11	33.33	-	44.44	100
nutrient density	F	15.38	7.69	7.69	30.77	69.2
	Total	13.63	18.18	4.55	36.36	81.82

Table 3.2 demonstrates that more than one-third of the subjects (36-45%) were correctly classified into the same quartile of dietary calcium intake by both methods. Ninety-one percent of the subjects were classified into the same or within-one quartile when express in terms of absolute value and 81.82% in terms of nutrient density.

There was no gross misclassification in males and females when expressed as either absolute value or nutrient densities. The correctly cross classification of calcium intake was about 38-56% and 31-44% for absolute value and nutrient density respectively.

The strongest consistency of nutrient intake estimated across dietary assessment method within one-quartile was observed in males whereas this consistency was lower in females when expressed as absolute value. The same finding was applied when expressed as nutrient density.

The difference between the two methods of dietary assessment in estimating mean intake of calcium was greater than 20% in adult<sup>(111)</sup> and higher than 50%<sup>(112)</sup> in children aged 4-5 years because the longer the recording period, the more declining in accuracy of recording which may attributed to fatigue and boredom and potential alterations of dietary habit. Although this questionnaire cannot estimate group mean intake as good as FR which represent as the reference method, the ability of the sFFQ to classify subjects according to their calcium was considered as an appropriate tool in our study because correct cross classification of 91% was significantly higher than the expected 65% correct cross classification due to chance alone.

Nelson and his colleague<sup>(113)</sup> examined the between and within subjects variation in nutrient intake which aimed to estimate the number of days required to rank dietary

intake with desired precision from all age groups. They reported that both within and between subject variation in each nutrient intake varied day to day and among subjects. Their finding revealed the range of number of days diet record required to estimate calcium intake were between 4-12 days to ensure perfect agreement for actual calcium intake ( $r \geq 0.09$ ). Hence, information obtained from 3 day dietary record may not represent their usual intake of this nutrient. Even though the use of sFFQ signified overestimation of actual intake, it revealed the good consistency in estimating the usual calcium intake in this study. Therefore, it can be concluded that the sFFQ for calcium intake designed for this study produced a valid estimates of mean dietary calcium intake in children subjects. The questionnaire was able to classify children into quartiles of calcium intake and had a high specificity in identifying children with high and low intake. However, the sFFQ from this study clearly cannot replace the diet record in estimating calcium intake for individual subject. The relevance of this sFFQ to other population is not our prime intention. Further validation studies with other population are encouraged.

### 3.5.3 Statistical Analysis

All measurement values in this study were reported as mean, standard deviation (SD), median, range and percentage. Statistical analysis focused on selected descriptive statistics: mean, SD, median, range and percentage. Before analyzed, all entry data was edited for any incomplete and extreme values. Kolmogorov-Smirnov Goodness of fit test was performed to determine the normality of the distribution of all variables. Statistical significance level was set at 0.05. Parametric statistics (student's t-test, analysis of variance) were applied to test the significance of differences between mean

values and nonparametric statistic (Mann Whitney U test) was also used for the non-normal distribution variables. Student's t-test was used to compare the difference of mean values between sex and Mann Whitney U test was used for non-normal distribution data. One way analysis of variance was used to determine the mean differences among four age groups: 1. 9.00-9.99 years, 2. 10.00-10.99 years, 3. 11.00-11.99 years, 4. 12.00-12.99 years with Scheffe's multiple range test was used to determine multiple comparisons. Using analysis of covariance to applied to determine the mean differences of bone parameters among pubertal stages by using age as covariate and multiple comparisons tests were made by Scheffe's procedure.

Pearson's product moment correlation was used to determine the relationship between each independent variables and dependent variable (bone values). Multiple regression was used to investigate the relationship between dependent variable and some independent variables with the effect of other independent variables statistically eliminated. The physiological and environmental factors were entered as independent variables and each bone values was entered as dependent variable. Residual plot were generated for each regression model to ensure that the assumption of multiple regression were not violated by the data itself. Sex and pubertal stages were coded as dummy variables as follows:

	dummy variable	
Sex	$X_1$	
male	0	
female	1	
Pubertal stage	$P_1$	$P_2$
pre-puberty	1	0
early-puberty	0	1
late-puberty	0	0

The reference category for sex is male and for pubertal stage is late-puberty.

Multiple regression was analyzed in 3 models by using each bone value as dependent variable. Model 1, physiological factors such as age, sex, weight, height, and pubertal stage were entered as independent variables. Model 2, environmental factors which were classified into 4 sub-models; model 2.1 usual calcium intake and weight bearing activity were entered as independent variables, model 2.2 current calcium intake and weight bearing activity were entered as independent variables; model 2.3 daily nutrient intake in absolute value were entered as independent variables; model 2.4 daily nutrient intake in terms of nutrient density were entered as independent variables. Model 3, all factors were entered simultaneously as independent variables.

This model was also subclassified into 2 sub-models; model 3.1 physiological factors, usual calcium intake and weight bearing activity; model 3.2 physiological factors and daily nutrient intake in absolute value.



## CHAPTER IV

### RESULTS

This study aimed to determine the bone mass and its related factors in healthy Thai children living in Bangkok.

#### 4.1 Demographic characteristics of the study subjects

A group of 276 children and adolescents, aged 9 to 12 years, was enrolled in this study. Two children were excluded from this study due to incomplete bone measurement. Therefore, the results in this study were based on 274 subjects with equal number for males and females (137 subjects in each group). Demographic characteristics of the subjects are shown in Table 4.1. According to age and sex, the number and percentage distribution in each group were similar. The mean age of males and females were equal,  $11.1 \pm 1.1$  years. The age onset of puberty in females was significantly different, about 1 year earlier from males ( $p=0.000$ ). About 82 percent of males, with equal distribution, was in pre-puberty (Tanner stage 1) and early-puberty (Tanner stage 2-3). Sixty-one percent of females was in early-puberty followed by 22% in late puberty and 17% in pre-puberty.

**Table 4.1** Demographic characteristics of the subjects classified by sex

Variable	Males (137)	Females (137)	Total (274) <sup>#</sup>
<b>Age (y)</b>			
9.00-9.99	9.7 ± 0.3 <sup>†</sup> (29)	9.6 ± 0.2 (27)	9.7 ± 0.3 (56) <sup>#</sup>
10.00-10.99	10.5 ± 0.3 (35)	10.4 ± 0.3 (38)	10.4 ± 0.3 (73)
11.00-11.99	11.5 ± 0.3 (39)	11.5 ± 0.3 (37)	11.5 ± 0.3 (76)
12.00-12.99	12.4 ± 0.2 (34)	12.4 ± 0.2 (35)	12.4 ± 0.2 (69)
<b>Puberty (%)</b>			
Pubertal stage 1	41 (56)	17 (23)	29 (79)
Pubertal stage 2	33 (45)	17 (23)	25 (68)
Pubertal stage 3	8 (11)	44 (61)	26 (72)
Pubertal stage 4	8 (11)	20 (27)	14 (38)
Pubertal stage 5	10 (14)	2 (3)	6 (17)
Age at onset of puberty (y)	11.3 ± 0.8	10.2 ± 0.6 <sup>**</sup>	10.9 ± 0.9
Age at menarche (y)	-	11.2 ± 0.8	-

<sup>#</sup> figure in the parentheses is number of subjects

<sup>†</sup> mean ± SD

<sup>\*\*</sup> significant difference between sex at P<0.01

**Table 4.2** Anthropometric parameters of the subjects classified by sex

<b>Anthropometric parameters</b>	<b>Males (137)</b>	<b>Females (137)</b>	<b>Total (274)<sup>#</sup></b>
Weight (kg)	36.9 ± 8.1 <sup>†</sup>	37.9 ± 7.6	37.4 ± 7.8
Height (cm)	144.0 ± 9.1	146.6 ± 8.1 <sup>*</sup>	145.3 ± 8.7
Weight for Age Z-score (%)			
- 1 SD to -2 SD	12 (17)	17 (23)	15 (40)
± 1 SD	76 (103)	77 (106)	76 (206)
> 1 SD to 2 SD	12 (17)	6 (8)	9 (25)
Height for Age Z-score (%)			
- 1 SD to -2 SD	10 (14)	6 (8)	8 (22)
± 1 SD	78 (107)	80 (110)	79 (217)
> 1 SD to 2 SD	12 (16)	14 (19)	13 (35)

<sup>#</sup> figure in the parentheses are number of subjects

<sup>†</sup> mean ± SD

<sup>\*</sup> significant difference between sex at P<0.05

### **Anthropometry**

Table 4.2 shows the anthropometric parameters of the study with mean body weight was  $36.9 \pm 8.1$  and  $37.9 \pm 7.6$  for males and females respectively. There was no difference in the mean values of body weight between males and females. Mean height of total subject was  $145.3 \pm 8.7$  cm of which females were significantly taller than males ( $146.6 \pm 8.1$  vs.  $144.0 \pm 9.1$ ,  $p=0.012$ ). Based on anthropometric reference data from NCHS (National Center for Health Statistics), the Z-score of weight for age and height for age, the subjects in this study had the score within the range of two standard deviations. Most of males and females had the score in the range of  $\pm 1$  SD (75% and 77% among males and females by weight for age and 78% and 77% among males and females by height for age, respectively).

### **4.2 Daily dietary and nutrient intake**

The average nutrient intakes of the subjects from 3-day food record are presented in Table 4.3. The mean calcium intake was  $530 \pm 241$  mg.d<sup>-1</sup> for males and  $492 \pm 219$  mg.d<sup>-1</sup> for females. Average sodium intake was  $2701 \pm 952$  mg.d<sup>-1</sup>, there was no significant difference in sex of daily calcium intake, sodium, and Ca:P ratio except for phosphorus of which males had significantly higher intake than females  $672 \pm 229$  vs.  $596 \pm 191$  mg.d<sup>-1</sup> ( $p=0.003$ ). When comparisons were made in terms of nutrient density (per 1000 kcal), there was no difference in the mean values of these nutrients between males and females. Males consumed more energy than females which resulting in higher mean values of all macronutrients. However, no statistical significant difference between the mean value of caloric distribution of carbohydrate,

**Table 4.3** Mean  $\pm$  SD of daily dietary intake from 3-day food record of the subjects classified by sex

Dietary intake	Males (136)	Females (136)	Total (272) <sup>#</sup>
Energy (kcal)	1713 $\pm$ 481	1522 $\pm$ 360 <sup>**</sup>	1617 $\pm$ 434
Carbohydrate (g.day <sup>-1</sup> )	209 $\pm$ 70	190 $\pm$ 54 <sup>*</sup>	200 $\pm$ 63
(%kcal)	49 $\pm$ 6	50 $\pm$ 7	49 $\pm$ 7
Protein (g.day <sup>-1</sup> )	72 $\pm$ 22	64 $\pm$ 17 <sup>*</sup>	68 $\pm$ 20
(%kcal)	17 $\pm$ 3	17 $\pm$ 3	17 $\pm$ 3
Fat (g.day <sup>-1</sup> )	65 $\pm$ 20	56 $\pm$ 17 <sup>*</sup>	60 $\pm$ 19
(%kcal)	34 $\pm$ 5	33 $\pm$ 6	33 $\pm$ 5
Calcium (mg.d <sup>-1</sup> )	530 $\pm$ 241	492 $\pm$ 219	511 $\pm$ 230
(mg.1000kcal <sup>-1</sup> )	315 $\pm$ 119	323 $\pm$ 123	319 $\pm$ 121
Phosphorus (mg.d <sup>-1</sup> )	672 $\pm$ 229	596 $\pm$ 191 <sup>*</sup>	634 $\pm$ 214
(mg.1000kcal <sup>-1</sup> )	395 $\pm$ 88	391 $\pm$ 84	393 $\pm$ 86
Ca : P ratio	1 : 1.5	1 : 1.4	1 : 1.5
Sodium (mg.d <sup>-1</sup> )	2792 $\pm$ 994	2609 $\pm$ 902	2701 $\pm$ 952
(mg.1000kcal <sup>-1</sup> )	1662 $\pm$ 486	1743 $\pm$ 546	1703 $\pm$ 517

<sup>#</sup> figures in the parentheses are number of subjects

<sup>\*</sup>, <sup>\*\*</sup> significant difference between sex at p<0.05, p<0.01 respectively

**Table 4.4** Mean  $\pm$  SD of calcium, phosphorus, protein, and sodium intake from 3-day food record of the subjects according to sex and age group

Dietary intake	Sex	Age group (y)				Total
		9.00 - 9.99	10.00 - 10.99	11.00 - 11.99	12.00 - 12.99	
Calcium (mg.d <sup>-1</sup> )	M	555 $\pm$ 238 (28)	575 $\pm$ 297 (35)	500 $\pm$ 192 (39)	499 $\pm$ 230 (34)	530 $\pm$ 241 (136) <sup>#</sup>
	F	517 $\pm$ 257 (27)	555 $\pm$ 196 (37)	464 $\pm$ 226 (37)	435 $\pm$ 189 (35)	492 $\pm$ 219 (136)
Phosphorus (mg.d <sup>-1</sup> )	M	667 $\pm$ 157	640 $\pm$ 249	622 $\pm$ 216	765 $\pm$ 252	672 $\pm$ 229
	F	584 $\pm$ 191	599 $\pm$ 228	595 $\pm$ 170	603 $\pm$ 177*	596 $\pm$ 191
Ca : P ratio	M	1 : 1.5	1 : 1.3	1 : 1.5	1 : 1.8	1 : 1.5
	F	1 : 1.4	1 : 1.2	1 : 1.5	1 : 1.7	1 : 1.4
Protein (g.d <sup>-1</sup> )	M	72 $\pm$ 20	69 $\pm$ 24	68 $\pm$ 22	80 $\pm$ 19	72 $\pm$ 22
	F	66 $\pm$ 19	64 $\pm$ 18	65 $\pm$ 17	63 $\pm$ 16*	64 $\pm$ 17
Sodium (mg.d <sup>-1</sup> )	M	2640 $\pm$ 775	2959 $\pm$ 1212	2487 $\pm$ 872	3095 $\pm$ 953	2792 $\pm$ 994
	F	2519 $\pm$ 851	2712 $\pm$ 1197	2643 $\pm$ 691	2536 $\pm$ 793*	2609 $\pm$ 902

<sup>#</sup> figures in the parentheses are number of subjects

\* significant difference between sex at p<0.05

**Table 4.5** Mean  $\pm$  SD of daily dietary calcium, phosphorus, protein and sodium intake from 3-day food record of the subjects classified by sex and pubertal stage

Dietary intake	Sex	Pubertal stage		
		Pre (79)	Early (140)	Late (55) <sup>#</sup>
Calcium (mg.d <sup>-1</sup> )	M	548 $\pm$ 263 (56)	523 $\pm$ 223 (55)	506 $\pm$ 233 (25) <sup>#</sup>
	F	505 $\pm$ 208 (23)	485 $\pm$ 212 (83)	499 $\pm$ 249 (30)
Phosphorus (mg.d <sup>-1</sup> )	M	639 $\pm$ 203	662 $\pm$ 239	767 $\pm$ 243
	F	568 $\pm$ 203	606 $\pm$ 192	590 $\pm$ 182
Ca : P	M	1 : 1.4	1 : 1.5	1 : 1.8
	F	1 : 1.3	1 : 1.5	1 : 1.5
Protein (g.d <sup>-1</sup> )	M	70 $\pm$ 22	71 $\pm$ 21	80 $\pm$ 22
	F	61 $\pm$ 20	65 $\pm$ 17	67 $\pm$ 15
Sodium (mg.d <sup>-1</sup> )	M	2679 $\pm$ 990	2783 $\pm$ 1010	3065 $\pm$ 953
	F	2607 $\pm$ 1063	2657 $\pm$ 923	2479 $\pm$ 702

<sup>#</sup> figures in the parentheses are number of subjects

protein and fat intake among sex was observed. The percentage of caloric distribution from carbohydrate, protein and fat between male and female subjects were 49 vs. 50, 17 vs. 17 and 34 vs. 33 respectively.

Table 4.4 shows mean values of calcium, phosphorus, protein and sodium intake from 3-day food record of the subjects according to sex and age group. Among different age groups, there was no significant difference in the consumption of daily calcium, phosphorus, Ca:P ratio, protein and sodium in each sex. However, in the age group of 12 years, males had statistically significant higher intake of phosphorus, protein and sodium than females.

As far as pubertal stage is concerned, after using the age variable as covariate in the analysis, daily consumption of calcium, phosphorus, Ca:P ratio, protein and sodium intake of males and females with different pubertal stages were not significantly different as displayed in Table 4.5.

On the basis of the current Thai RDA for calcium in this age group (800-1200 mg.d<sup>-1</sup>), the subjects were divided into 4 groups according to their calcium intake level ; <400 mg.d<sup>-1</sup>, 400-800 mg.d<sup>-1</sup>, 800-1200 mg.d<sup>-1</sup> and >1200 mg.d<sup>-1</sup> as shown in Table 4.6. Twenty-seven of (10%) 272 subjects consumed daily dietary calcium more than 800 mg up to 1200 mg which indicate an adequate calcium intake for their requirement. About half (56%) and one-third (33%) of total subjects had calcium intake within the range of 400-800 mg.d<sup>-1</sup> and less than 400 mg.d<sup>-1</sup>, respectively. Only three males (1%) consumed dietary calcium above the recommended value (>1200 mg.d<sup>-1</sup>).

Table 4.7 demonstrates number and percentage distribution of the subjects classified by sex and level of dietary calcium intake which derived from

**Table 4.6** Number and percentage distribution of the subjects classified by sex and level of dietary calcium intake from 3-day food record according to the RDA recommendation

Calcium intake (mg.d <sup>-1</sup> )	Males (136)	Females (136)	Total (272) <sup>#</sup>
<400	42 (31)	49 (36)	91 (33)
400 - 800	78 (57)	73 (54)	151 (56)
>800 - 1200	13 (10)	14 (10)	27 (10)
>1200	3 (2)	-	3 (1)

<sup>#</sup> figures in the parentheses are number of subjects

**Table 4.7** Number and percentage distribution of the subjects classified by sex and level of dietary calcium intake from semiquantitative food frequency questionnaire according to the RDA recommendation

Calcium intake (mg.d <sup>-1</sup> )	Males (136)	Females (137)	Total (273) <sup>#</sup>
<400	27 (20)	26 (19)	53 (19)
400 - 800	66 (48)	72 (53)	138 (50)
>800 - 1200	36 (27)	32 (23)	68 (25)
>1200	7 (5)	7 (5)	14 (6)

<sup>#</sup> figures in the parentheses are number of subjects

semiquantitative food frequency questionnaire (sFFQ) according to the RDA recommendation. The result revealed that half of total subject had daily dietary calcium intake in the range of 400-800mg.d<sup>-1</sup>. One quarter of total subject had reach the RDA recommendation for this age group (>800-1200 mg.d<sup>-1</sup>). Nineteen percent of total subject consumed calcium intake less than 400 mg.d<sup>-1</sup>. Only fourteen subjects had greater calcium intake than recommended value.

#### *Usual calcium intake and food sources*

To identify the food sources and their usual intake of calcium in the subjects, the results derived from sFFQ were determined as shown in Table 4.8. The mean value of usual calcium intake were  $667 \pm 281$  (range 162-1551) mg.d<sup>-1</sup> and  $671 \pm 302$  (range 70-1601) mg.d<sup>-1</sup> for males and females, respectively. There was no significant difference in calcium intake among males and females. Major food source for calcium intake in this subjects was milk and milk product group which contributed to more than half (57%) of their daily calcium intake. The second important food group was fish and aquatic animals which contributed to approximately 12% of total calcium intake. The other food groups that contributed to only 5-6% of total calcium intake were vegetables, cereal, snack and dessert groups. These four latter food groups contributed to 28% of calcium intake.

**Table 4.8** Mean  $\pm$  SD and the percentage contribution from various food groups of daily dietary calcium intake of subjects according to sex

Food group	Male		Female		Total	
	Calcium (mg.d <sup>-1</sup> )	% calcium distribution	Calcium (mg.d <sup>-1</sup> )	% calcium distribution	Calcium (mg.d <sup>-1</sup> )	% calcium distribution
<b>Milk &amp; milk products</b>	392 $\pm$ 227	57	382 $\pm$ 247	56	387 $\pm$ 237	57
<b>Fish and aquatic animal</b>	73 $\pm$ 61	11	88 $\pm$ 86	13	80 $\pm$ 75	12
- fish with soft bone	25 $\pm$ 33	4	31 $\pm$ 41	5	28 $\pm$ 37	4
- lean fish	28 $\pm$ 37	4	24 $\pm$ 30	3	26 $\pm$ 34	4
- seafood (fresh & dried)	20 $\pm$ 29	3	32 $\pm$ 50	5	26 $\pm$ 42	4
<b>Meat, egg &amp; products</b>	27 $\pm$ 18	4	26 $\pm$ 16	4	26 $\pm$ 17	4
<b>Cereal, grains and products</b>	40 $\pm$ 18	6	33 $\pm$ 14	5	36 $\pm$ 16	5
<b>Legumes and products</b>	17 $\pm$ 19	3	20 $\pm$ 26	3	19 $\pm$ 23	3
<b>Vegetables</b>	32 $\pm$ 25	5	40 $\pm$ 39	6	36 $\pm$ 33	5
<b>Fruits</b>	14 $\pm$ 14	2	17 $\pm$ 18	2	16 $\pm$ 16	2
<b>Fast food</b>	16 $\pm$ 16	2	13 $\pm$ 14	2	14 $\pm$ 15	2
<b>Snack and dessert</b>	42 $\pm$ 30	6	40 $\pm$ 33	6	41 $\pm$ 31	6
- Thai style	29 $\pm$ 26	4	27 $\pm$ 27	4	28 $\pm$ 27	4
- Western style	14 $\pm$ 10	2	13 $\pm$ 10	2	13 $\pm$ 10	2
<b>Miscellaneous</b>	29 $\pm$ 30	4	27 $\pm$ 32	4	28 $\pm$ 31	4
- beverage	27 $\pm$ 30	4	26 $\pm$ 32	4	27 $\pm$ 31	4
- others	1 $\pm$ 1	-	1 $\pm$ 1	-	1 $\pm$ 1	-
<b>Total calcium intake</b>	667 $\pm$ 281	-	671 $\pm$ 302	-	669 $\pm$ 291	-

### 4.3 Factors affecting bone mass

#### 4.3.1 Age and gender

Age and gender have been considered as major determinants of bone mass. Therefore, the children subjects were stratified by age and sex and the statistical analysis was performed to determine the difference of their bone mass values.

##### *Bone mineral density*

When comparison was made among different age groups, as shown in Table 4.9 and Figure 1, it was found that the accumulation of BMD was significantly pronounced at the age of 11 years in females and at the age of 12 years in males. Among males, only femoral neck BMD did not increase with advancing age whereas in females, midshaft radius BMD increase at the age of 12 years. For volumetric BMD, an increment of this value at all sites with advancing age was not observed in males but there was markedly increased at lumbar spine in females by the age of 12 years.

Among the 9 year-old group, males had significantly higher mean BMD values than females at femoral neck ( $p=0.04$ ). Females had significantly higher BMD than males at lumbar spine in all age groups ( $0.000 < p < 0.003$ ), at total body BMD among the 10 and 11 year-old group ( $p=0.25$ ,  $p=0.015$ , respectively) as well as at midshaft radius in 10 year-old age group ( $p=0.000$ ). Females also had significantly greater mean values of vBMD than males at lumbar spine in all age groups ( $0.000 < p < 0.001$ ) and at femoral neck in the age group of 11 years old ( $p=0.20$ ).

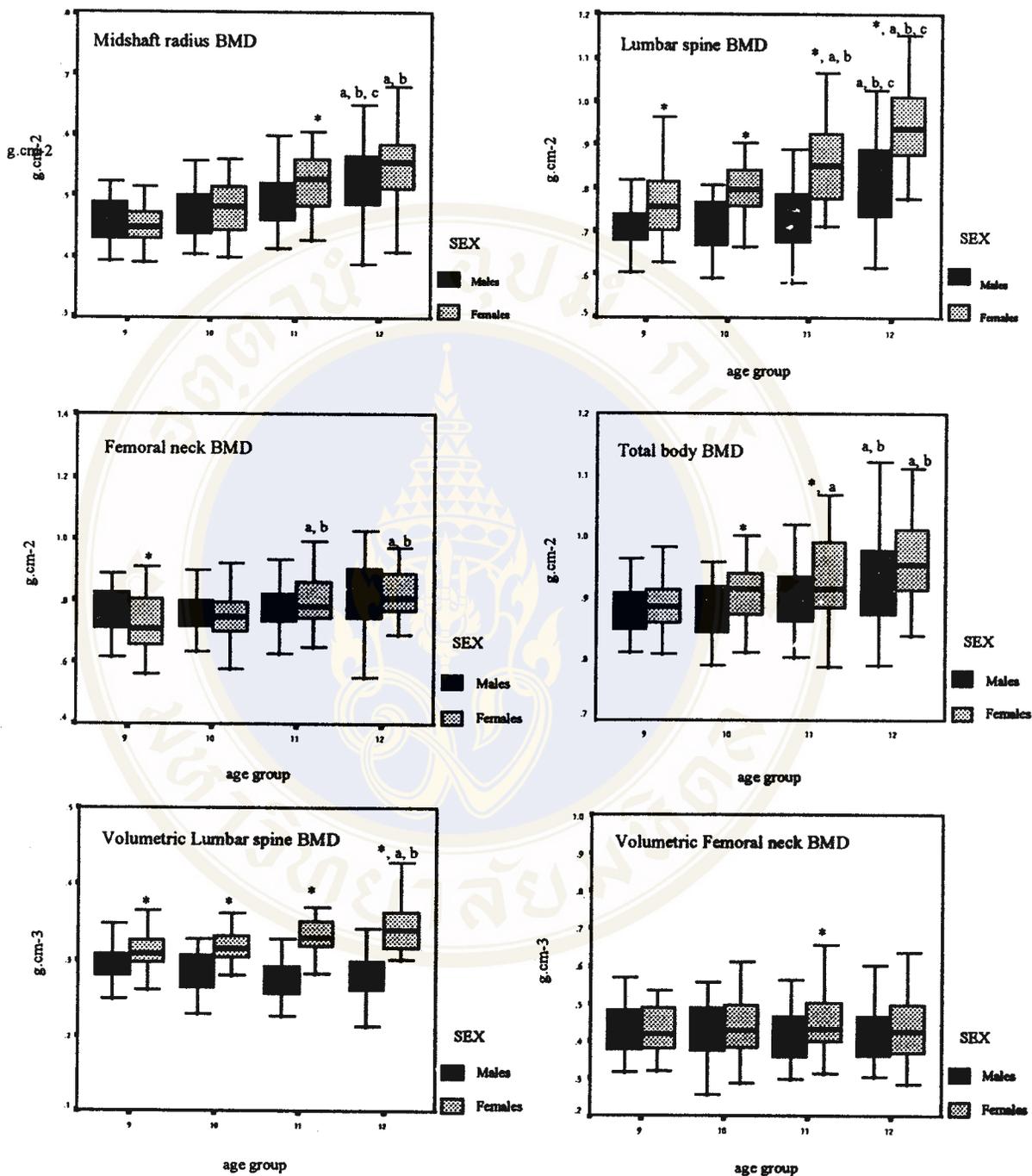
##### *Bone mineral content*

The relationship between age and bone mineral content at the three bone studied sites are shown in Table 4.10 and Fig. 2. An increase in the BMC values at all sites

**Table 4.9** Mean  $\pm$  SD of areal bone mineral density and volumetric BMD at midshaft radius, lumbar spine, femoral neck and total body of the subjects according to sex and age group

Bone mineral density	Sex	Age group (y)					Total
		9.00 - 9.99	10.00 - 10.99	11.00 - 11.99	12.00 - 12.99		
Midshaft radius (g.cm <sup>-2</sup> )	M	0.46 $\pm$ 0.04 <sup>a</sup> (29) <sup>#</sup>	0.47 $\pm$ 0.04 <sup>a</sup> (35)	0.49 $\pm$ 0.04 <sup>a</sup> (39)	0.53 $\pm$ 0.07 <sup>b</sup> (34)	0.49 $\pm$ 0.06 (137)	
	F	0.45 $\pm$ 0.04 <sup>a</sup> (27)	0.48 $\pm$ 0.05 <sup>a</sup> (38)	0.52 $\pm$ 0.05 <sup>a,b,*</sup> (37)	0.55 $\pm$ 0.07 <sup>b</sup> (35)	0.50 $\pm$ 0.06 <sup>*</sup> (137)	
Lumbar spine (g.cm <sup>-2</sup> )	M	0.71 $\pm$ 0.06 <sup>a</sup>	0.71 $\pm$ 0.06 <sup>a</sup>	0.73 $\pm$ 0.08 <sup>a</sup>	0.81 $\pm$ 0.10 <sup>b</sup>	0.74 $\pm$ 0.09	
	F	0.77 $\pm$ 0.08 <sup>a,*</sup>	0.79 $\pm$ 0.07 <sup>a,*</sup>	0.86 $\pm$ 0.10 <sup>b,*</sup>	0.94 $\pm$ 0.09 <sup>c,*</sup>	0.85 $\pm$ 0.11 <sup>*</sup>	
Femoral neck (g.cm <sup>-2</sup> )	M	0.79 $\pm$ 0.12 <sup>a</sup>	0.76 $\pm$ 0.08 <sup>a</sup>	0.78 $\pm$ 0.08 <sup>a</sup>	0.82 $\pm$ 0.13 <sup>a</sup>	0.80 $\pm$ 0.11	
	F	0.73 $\pm$ 0.09 <sup>a,*</sup>	0.74 $\pm$ 0.09 <sup>a</sup>	0.81 $\pm$ 0.10 <sup>b</sup>	0.82 $\pm$ 0.09 <sup>b</sup>	0.78 $\pm$ 0.10	
Total body (g.cm <sup>-2</sup> )	M	0.88 $\pm$ 0.04 <sup>a</sup>	0.88 $\pm$ 0.05 <sup>a</sup>	0.90 $\pm$ 0.05 <sup>a,b</sup>	0.93 $\pm$ 0.08 <sup>b</sup>	0.90 $\pm$ 0.06	
	F	0.89 $\pm$ 0.06 <sup>a</sup>	0.91 $\pm$ 0.05 <sup>a,b,*</sup>	0.93 $\pm$ 0.07 <sup>b,c,*</sup>	0.96 $\pm$ 0.07 <sup>c</sup>	0.92 $\pm$ 0.07 <sup>*</sup>	
Volumetric lumbar spine (g.cm <sup>-3</sup> )	M	0.29 $\pm$ 0.02 <sup>a</sup>	0.28 $\pm$ 0.03 <sup>a</sup>	0.28 $\pm$ 0.03 <sup>a</sup>	0.28 $\pm$ 0.03 <sup>a</sup>	0.28 $\pm$ 0.03	
	F	0.31 $\pm$ 0.02 <sup>a,*</sup>	0.32 $\pm$ 0.02 <sup>a,*</sup>	0.33 $\pm$ 0.02 <sup>a,b,*</sup>	0.34 $\pm$ 0.03 <sup>b,*</sup>	0.33 $\pm$ 0.03 <sup>*</sup>	
Volumetric femoral neck (g.cm <sup>-3</sup> )	M	0.44 $\pm$ 0.11 <sup>a</sup>	0.44 $\pm$ 0.10 <sup>a</sup>	0.41 $\pm$ 0.08 <sup>a</sup>	0.41 $\pm$ 0.08 <sup>a</sup>	0.43 $\pm$ 0.09	
	F	0.43 $\pm$ 0.09 <sup>a</sup>	0.43 $\pm$ 0.09 <sup>a</sup>	0.45 $\pm$ 0.08 <sup>a,*</sup>	0.44 $\pm$ 0.10 <sup>a</sup>	0.44 $\pm$ 0.09	

<sup>#</sup> figure in the parentheses are number of subjects  
<sup>a, b, c</sup> mean within the same row with different superscript are significantly difference (p<0.05)  
<sup>\*</sup> significant difference between sex at p<0.05



**Figure 1** Boxplot of areal bone mineral density (g.cm<sup>-2</sup>) and volumetric bone mineral density (g.cm<sup>-3</sup>) against age group for males and females at midshaft radius, lumbar spine, femoral neck and total body. The top and bottom of each box represent the 75<sup>th</sup> and 25<sup>th</sup> percentile respectively; and the horizontal bar within each box represent the median value for the appropriate segment of the studied subjects.

\* significant difference between sex;

a, b, c : significant difference from age group 9, 10 and 11 respectively

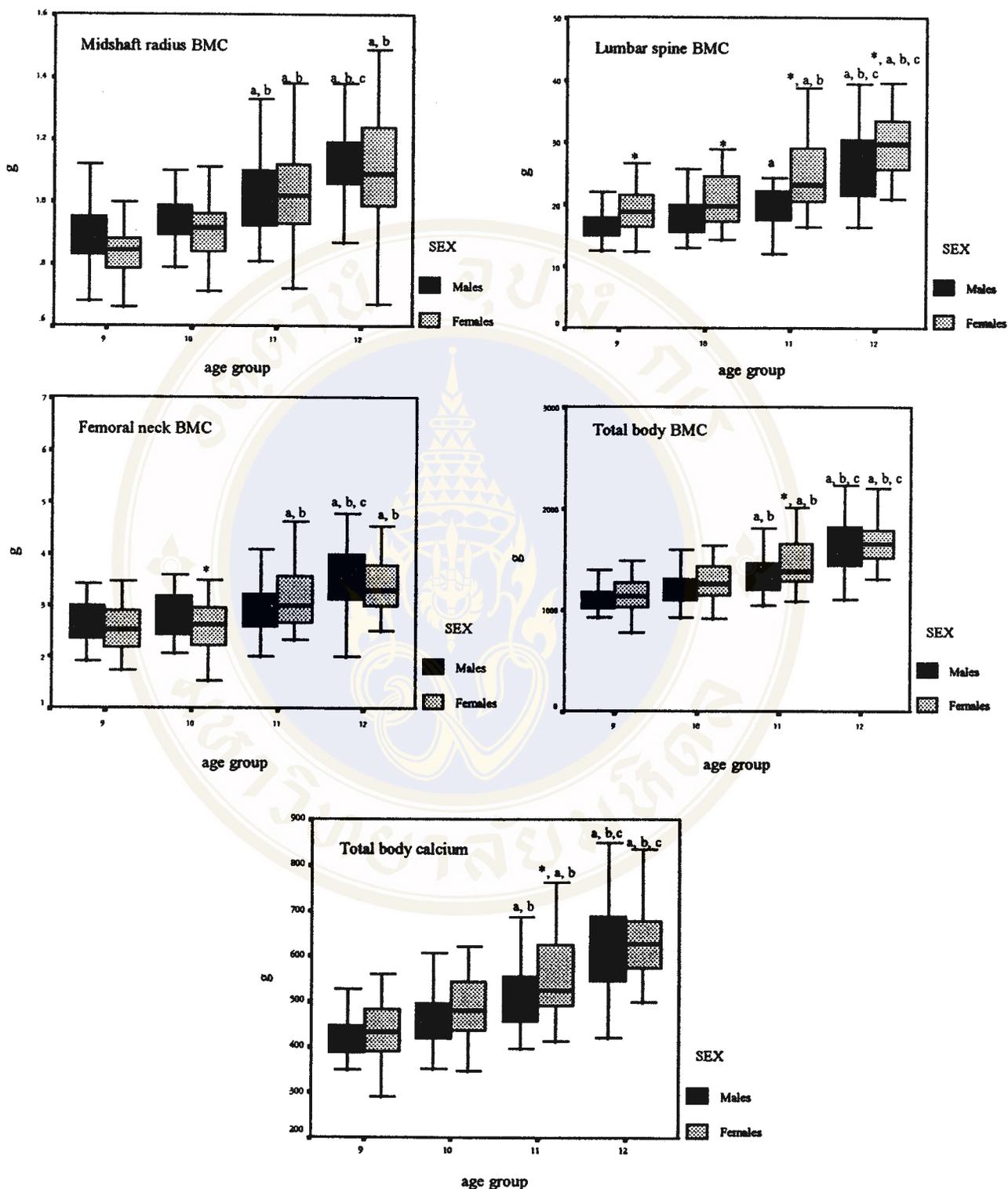
**Table 4.10** Mean  $\pm$  SD of bone mineral content at midshaft radius, lumbar spine, femoral neck, total body and total body calcium of the subjects according to sex and age group

Bone mineral content	Sex	Age group					Total
		9.00 - 9.99	10.00 - 10.99	11.00 - 11.99	12.00 - 12.99		
Midshaft radius (g)	M	0.90 $\pm$ 0.09 <sup>a</sup> (29)	0.95 $\pm$ 0.10 <sup>a, b</sup> (35)	1.01 $\pm$ 0.13 <sup>b</sup> (39)	1.13 $\pm$ 0.14 <sup>c</sup> (34)	1.00 $\pm$ 0.14 (137) <sup>#</sup>	
	F	0.85 $\pm$ 0.10 <sup>a</sup> (27)	0.91 $\pm$ 0.10 <sup>a</sup> (38)	1.03 $\pm$ 0.15 <sup>b</sup> (37)	1.11 $\pm$ 0.17 <sup>b</sup> (35)	0.98 $\pm$ 0.17 (137)	
Lumbar Spine (g)	M	16.62 $\pm$ 2.41 <sup>a</sup>	17.86 $\pm$ 2.78 <sup>a, b</sup>	19.85 $\pm$ 3.70 <sup>b</sup>	26.54 $\pm$ 5.99 <sup>c</sup>	20.38 $\pm$ 5.52	
	F	19.17 $\pm$ 3.98 <sup>a, *</sup>	20.80 $\pm$ 4.12 <sup>a, *</sup>	24.83 $\pm$ 5.62 <sup>b, *</sup>	30.00 $\pm$ 5.76 <sup>c, *</sup>	23.90 $\pm$ 6.43 <sup>*</sup>	
Femoral Neck (g)	M	2.77 $\pm$ 0.66 <sup>a</sup>	2.93 $\pm$ 0.72 <sup>a</sup>	2.99 $\pm$ 0.52 <sup>a</sup>	3.54 $\pm$ 0.80 <sup>b</sup>	3.07 $\pm$ 0.73	
	F	2.55 $\pm$ 0.47 <sup>a</sup>	2.57 $\pm$ 0.48 <sup>a, *</sup>	3.13 $\pm$ 0.55 <sup>b</sup>	3.34 $\pm$ 0.49 <sup>b</sup>	2.92 $\pm$ 0.60	
Total body (g)	M	1099 $\pm$ 116 <sup>a</sup>	1212 $\pm$ 160 <sup>a</sup>	1354 $\pm$ 212 <sup>b</sup>	1645 $\pm$ 288 <sup>c</sup>	1336 $\pm$ 286	
	F	1164 $\pm$ 200 <sup>a</sup>	1275 $\pm$ 181 <sup>a</sup>	1474 $\pm$ 234 <sup>b, *</sup>	1679 $\pm$ 245 <sup>c</sup>	1410 $\pm$ 288 <sup>*</sup>	
Total body calcium (g)	M	417 $\pm$ 44 <sup>a</sup>	461 $\pm$ 61 <sup>a</sup>	514 $\pm$ 80 <sup>b</sup>	625 $\pm$ 109 <sup>c</sup>	508 $\pm$ 109	
	F	442 $\pm$ 76 <sup>a</sup>	484 $\pm$ 69 <sup>a</sup>	560 $\pm$ 89 <sup>b, *</sup>	638 $\pm$ 93 <sup>c</sup>	536 $\pm$ 109 <sup>*</sup>	

<sup>#</sup> figure in the parentheses are number of subjects

<sup>a, b, c</sup> mean within the same row with different superscript are significantly difference (p<0.05)

<sup>\*</sup> significant difference between sex at p<0.05



**Figure 2** Boxplot of bone mineral content (g) against age group for males and females at midshaft radius, lumbar spine, femoral neck, total body and total body calcium. The top and bottom of each box represent the 75<sup>th</sup> and 25<sup>th</sup> percentile respectively; and the horizontal bar within each box represent the median value for the appropriate segment of the study subjects.

\* significant difference between sex;

a, b, c : significant difference from age group 9, 10 and 11 respectively

with advancing age was similar in both males and females as well as the value of total body BMC and total body calcium by the age of 11 years old. The accumulation of BMC at femoral neck was delayed by one year comparing to the other bone values.

Males had significantly higher femoral neck BMC value than females at the age of 10 years ( $p=0.001$ ). Females had significantly greater BMC at lumbar spine in all age groups than males ( $0.000 < p < 0.017$ ). The significant difference of BMC at midshaft radius between males and females was not observed. Total body BMC and TBC had statistically significant difference between males and females at the age of 11 years ( $p=0.022$ ).

#### **4.3.2 Puberty**

To determine the effect of stage of sexual maturation on bone parameters, the subjects were categorized into 3 groups according to their pubertal status: pre-puberty (Tanner stage 1), early-puberty (Tanner stage 2-3) and late-puberty (Tanner stage 4-5). It is recognized that age had an effect on pubertal development and the results from previous section indicated a significant effect on bone parameter at various bone sites. Therefore, to determine the effect of puberty on bone status, the analysis of covariance using age as covariate was performed. The result revealed the significant effect of puberty by exhibiting a higher mean of bone mass in pubertal children.

##### *Bone mineral density*

The mean values of BMD with different pubertal stages are displayed in Table 4.11. The mean of BMD was markedly increased in late-puberty as compare to pre- and early-puberty for both males and females as shown in Fig 3. Among males, there were no significant difference in the mean values of aBMD between pre- and early-

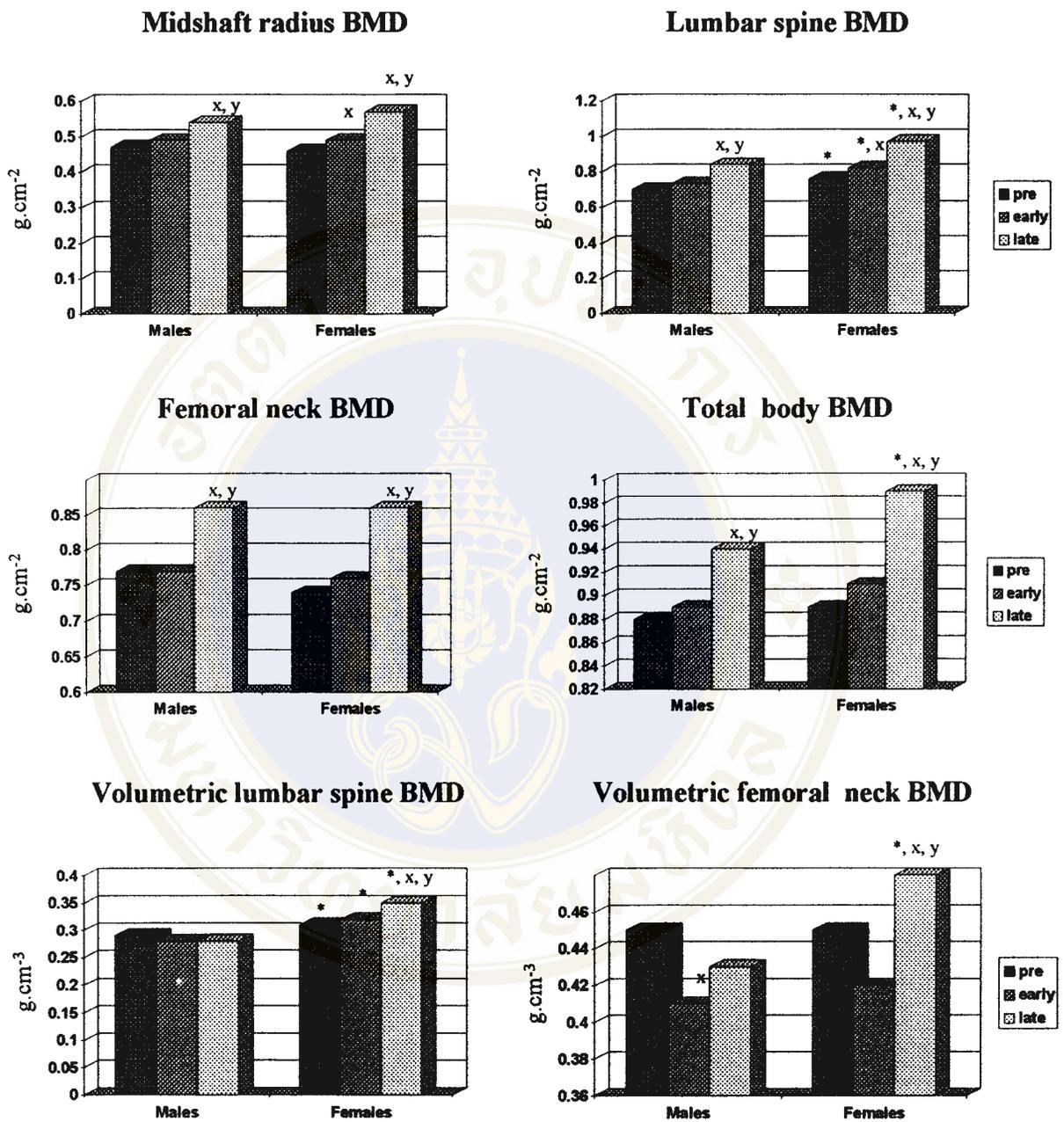
**Table 4.11** Mean  $\pm$  SD of areal bone mineral density and volumetric bone mineral density at midshaft radius, lumbar spine, femoral neck and total body of the subjects classified by sex and pubertal stage

Bone mineral density	Sex	Pubertal stage		
		Pre (79)	Early (140)	Late (55) <sup>#</sup>
Midshaft radius (g.cm <sup>-2</sup> )	M	0.47 $\pm$ 0.04 <sup>a</sup> (56)	0.49 $\pm$ 0.05 <sup>a</sup> (56)	0.54 $\pm$ 0.06 <sup>b</sup> (25) <sup>#</sup>
	F	0.46 $\pm$ 0.05 <sup>a</sup> (23)	0.49 $\pm$ 0.05 <sup>b</sup> (84)	0.57 $\pm$ 0.06 <sup>c</sup> (30)
Lumbar spine (g.cm <sup>-2</sup> )	M	0.70 $\pm$ 0.06 <sup>a</sup>	0.73 $\pm$ 0.08 <sup>a</sup>	0.84 $\pm$ 0.09 <sup>b</sup>
	F	0.76 $\pm$ 0.05 <sup>a,*</sup>	0.82 $\pm$ 0.09 <sup>b,*</sup>	0.97 $\pm$ 0.09 <sup>c,*</sup>
Femoral neck (g.cm <sup>-2</sup> )	M	0.77 $\pm$ 0.11 <sup>a</sup>	0.77 $\pm$ 0.08 <sup>a</sup>	0.86 $\pm$ 0.12 <sup>b</sup>
	F	0.74 $\pm$ 0.09 <sup>a</sup>	0.76 $\pm$ 0.09 <sup>a</sup>	0.86 $\pm$ 0.10 <sup>b</sup>
Total body (g.cm <sup>-2</sup> )	M	0.88 $\pm$ 0.05 <sup>a</sup>	0.89 $\pm$ 0.05 <sup>a</sup>	0.94 $\pm$ 0.08 <sup>b</sup>
	F	0.89 $\pm$ 0.05 <sup>a</sup>	0.91 $\pm$ 0.06 <sup>a</sup>	0.99 $\pm$ 0.06 <sup>b,*</sup>
Volumetric lumbar spine (g.cm <sup>-3</sup> )	M	0.29 $\pm$ 0.03	0.28 $\pm$ 0.03	0.28 $\pm$ 0.03
	F	0.31 $\pm$ 0.03 <sup>a,*</sup>	0.32 $\pm$ 0.03 <sup>a,*</sup>	0.35 $\pm$ 0.03 <sup>b,*</sup>
Volumetric femoral neck (g.cm <sup>-3</sup> )	M	0.45 $\pm$ 0.11 <sup>b</sup>	0.41 $\pm$ 0.07 <sup>a</sup>	0.43 $\pm$ 0.08 <sup>a,b</sup>
	F	0.45 $\pm$ 0.08 <sup>a</sup>	0.42 $\pm$ 0.08 <sup>a</sup>	0.48 $\pm$ 0.09 <sup>b,*</sup>

<sup>#</sup> figures in the parentheses are number of subjects

<sup>a, b, c</sup> mean within the same row with different superscript are significant difference (p<0.05)

\* significant difference between sex at p<0.05



**Figure 3** Mean  $\pm$  SD bone mineral density (g.cm<sup>-2</sup>) and volumetric bone mineral density (g.cm<sup>-3</sup>) for males and females at midshaft radius, lumbar spine, femoral neck and total body according to sex and pubertal stage.

\* significant difference between sex in the same pubertal stage at p<0.05

x, y : significant difference from pre-puberty and early-puberty respectively

puberty at all bone sites as well as the value of total body BMD. Among females, the significant differences in mean values of aBMD between pre- and early-puberty were found at midshaft radius ( $p=0.011$ ) and lumbar spine ( $p=0.003$ ). The significant differences in the mean values of BMD between pre- or early-puberty and late-puberty were observed at all bone sites and total body BMD for both males ( $0.000 < p < 0.003$ ) and females ( $0.000 < p < 0.011$ ).

For volumetric BMD, the accumulation of BMD at lumbar spine did not increase with advancing pubertal stage among males whereas the significant difference between pre- or early-puberty and late-puberty was observed at lumbar spine ( $p=0.000$ ) and femoral neck ( $p=0.004$ ) among females. Interestingly, the mean value of volumetric BMD at femoral neck were significantly decreased at early-puberty for males while similar finding was also observed among females but the value did not reach statistical significance.

Females had significantly greater mean values of BMD for both areal and volumetric BMD at lumbar spine than males at all Tanner stages ( $0.000 < p < 0.001$ ). The significant difference in bone value between sex was observed at late puberty for total body BMD ( $p=0.013$ ), femoral neck vBMD ( $p=0.019$ ). There were no difference in the mean values between males and females for midshaft radius and femoral neck at any Tanner stage.

#### *Bone mineral content*

The mean values of BMC at all study bone sites are shown in Table 4.12 and figure 4. A similar manner of an increment of BMC with advancing pubertal stage among males and females was observed. Among males, there were statistically

**Table 4.12** Mean  $\pm$  SD of bone mineral content at midshaft radius, lumbar spine, femoral neck, total body BMC and total body calcium of the subjects classified by sex and pubertal stage

Bone mineral content	Sex	Pubertal stage		
		Pre (79)	Early (140)	Late (55) <sup>#</sup>
Midshaft radius (g)	M	0.91 $\pm$ 0.10 <sup>a</sup> (56)	1.02 $\pm$ 0.13 <sup>b</sup> (56)	1.15 $\pm$ 0.13 <sup>c</sup> (25) <sup>#</sup>
	F	0.86 $\pm$ 0.08 <sup>a*</sup> (23)	0.95 $\pm$ 0.13 <sup>b*</sup> (84)	1.15 $\pm$ 0.17 <sup>c</sup> (30)
Lumbar spine (g)	M	17.2 $\pm$ 2.9 <sup>a</sup>	20.0 $\pm$ 3.63 <sup>b</sup>	28.4 $\pm$ 5.73 <sup>c</sup>
	F	18.0 $\pm$ 2.4 <sup>a</sup>	23.0 $\pm$ 4.90 <sup>b*</sup>	31.2 $\pm$ 6.02 <sup>c</sup>
Femoral neck (g)	M	2.85 $\pm$ 0.74 <sup>a</sup>	2.96 $\pm$ 0.47 <sup>a</sup>	3.78 $\pm$ 0.76 <sup>b</sup>
	F	2.50 $\pm$ 0.42 <sup>a*</sup>	2.83 $\pm$ 0.53 <sup>b</sup>	3.48 $\pm$ 0.52 <sup>c</sup>
Total body (g)	M	1150 $\pm$ 146 <sup>a</sup>	1349 $\pm$ 222 <sup>b</sup>	1724 $\pm$ 253 <sup>c</sup>
	F	1143 $\pm$ 133 <sup>a</sup>	1370 $\pm$ 220 <sup>b</sup>	1737 $\pm$ 269 <sup>c</sup>
Total body calcium (g)	M	437 $\pm$ 56 <sup>a</sup>	513 $\pm$ 84 <sup>b</sup>	655 $\pm$ 96 <sup>c</sup>
	F	434 $\pm$ 51 <sup>a</sup>	521 $\pm$ 84 <sup>b</sup>	656 $\pm$ 102 <sup>c</sup>

<sup>#</sup> figure in the parentheses are number of subjects  
<sup>a, b, c</sup> mean within the same row with different superscript are significant difference (p<0.05)  
<sup>\*</sup> significant difference between sex at p<0.05

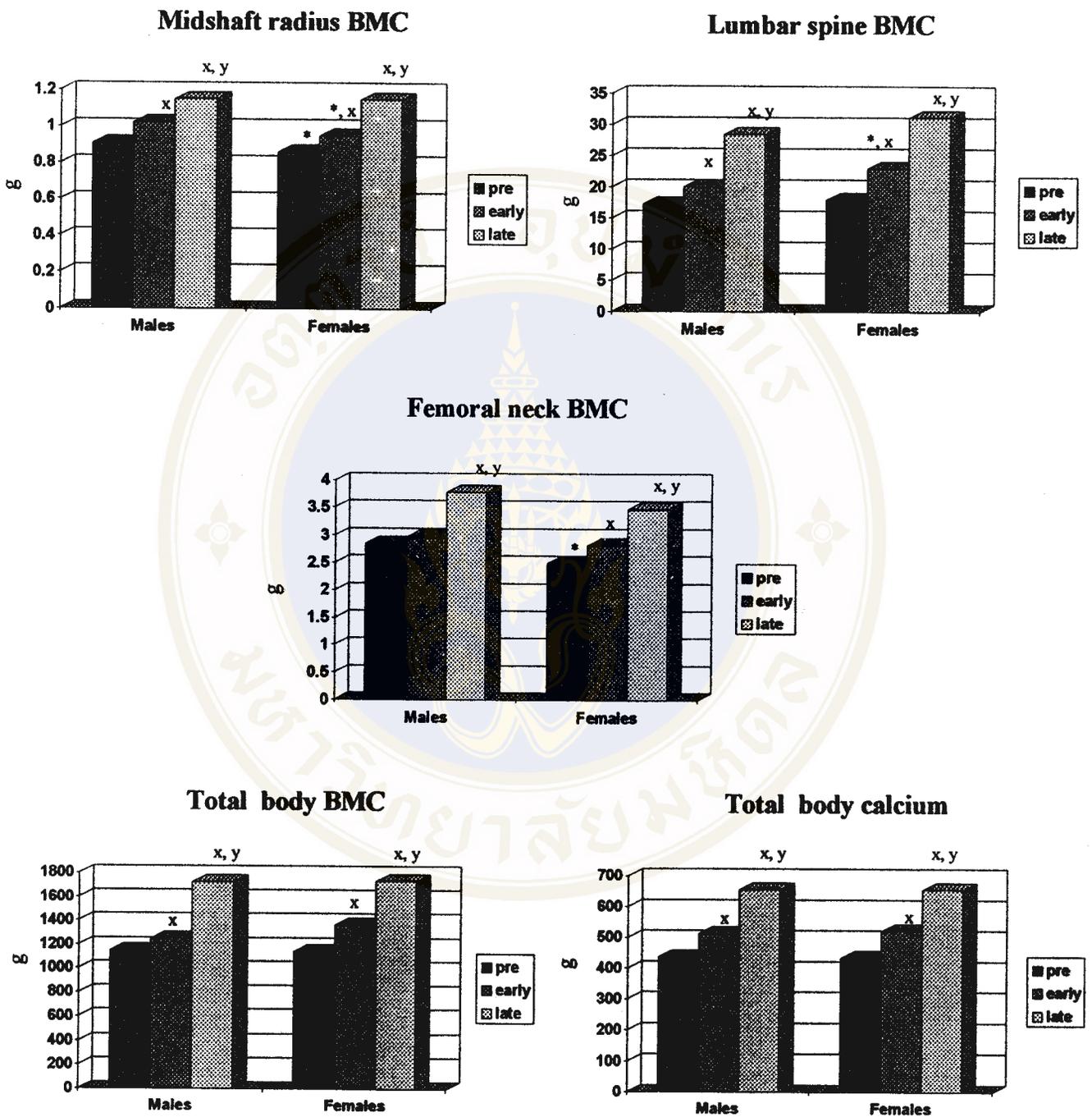


Figure 4 Mean  $\pm$  SD bone mineral content (g) for males and females at midshaft of radius, lumbar spine, femoral neck, total body BMC and total body calcium according to sex and pubertal stage.

\* significant difference between sex in the same pubertal stage at  $p < 0.05$

x, y : significant difference from pre-puberty and early-puberty respectively

significant difference between the mean BMC values of pre- and early-puberty at midshaft radius ( $p=0.000$ ), lumbar spine ( $p=0.001$ ), total body BMC ( $p=0.000$ ) and total body calcium ( $p=0.000$ ) except for femoral neck. Among females, there were statistically significant difference between the mean values of pre- and early-puberty in all BMC values and total body calcium ( $0.000 < p < 0.025$ ). When we considered all children subject without regarding their sex, the significant differences in total body BMC and the BMC at all bone sites between pre- or early and late-puberty including total body calcium were observed ( $0.000 < p < 0.025$ ).

Among the pre-pubertal group, males had greater mean values of BMC than females at midshaft radius ( $p=0.027$ ), and femoral neck ( $p=0.034$ ). Early-pubertal males had greater mean value of BMC at midshaft radius ( $p=0.003$ ) whereas early-pubertal females had greater mean values of BMC at lumbar spine ( $p=0.000$ ). At late-puberty, there was no difference in mean values of BMC in both males and females.

#### **4.3.3 Additive effect of usual dietary calcium intake**

Results from previous section clearly indicated the significant effect of pubertal stage on bone parameters of both total body and at specific bone sites. In this section, we further determine the additive effect of dietary calcium on bone parameters by categorizing the subjects in each sex and each pubertal stage, into 2 groups according to the level of their usual dietary calcium intake (below and above  $669 \text{ mg}\cdot\text{d}^{-1}$  which was the mean calcium intake of the group).

##### *Bone mineral density*

The mean values of BMD of the study subject as classified by sex, pubertal stage and level of calcium intake are shown in Table 4.13. There were significantly greater

**Table 4.13** Mean  $\pm$  SD of bone mineral density classified by level of calcium intake from FFQ, sex and pubertal stage of the subjects.

Bone mineral density	Sex	Pre-puberty		Early-puberty		Late-puberty	
		$\leq 669$ (mg.d <sup>-1</sup> )	>669 (mg.d <sup>-1</sup> )	$\leq 669$ (mg.d <sup>-1</sup> )	>669 (mg.d <sup>-1</sup> )	$\leq 669$ (mg.d <sup>-1</sup> )	>669 (mg.d <sup>-1</sup> )
Midshaft of radius (g.cm <sup>-2</sup> )	M	0.46 $\pm$ 0.03 (31)	0.47 $\pm$ 0.04 (25)	0.49 $\pm$ 0.06 (35)	0.49 $\pm$ 0.04 (20)	0.52 $\pm$ 0.05 (13)	0.56 $\pm$ 0.06 (12) <sup>#</sup>
	F	0.44 $\pm$ 0.02 (7)	0.47 $\pm$ 0.05 (16)	0.49 $\pm$ 0.05 (49)	0.50 $\pm$ 0.05 (35)	0.57 $\pm$ 0.04 (17)	0.56 $\pm$ 0.07 (13)
Lumbar spine (g.cm <sup>-2</sup> )	M	0.70 $\pm$ 0.07	0.71 $\pm$ 0.06	0.73 $\pm$ 0.08	0.74 $\pm$ 0.08	0.82 $\pm$ 0.10	0.85 $\pm$ 0.09
	F	0.73 $\pm$ 0.05	0.77 $\pm$ 0.05	0.82 $\pm$ 0.09	0.83 $\pm$ 0.08	0.99 $\pm$ 0.09	0.95 $\pm$ 0.09
Femoral neck (g.cm <sup>-2</sup> )	M	0.75 $\pm$ 0.08	0.80 $\pm$ 0.13	0.77 $\pm$ 0.09	0.78 $\pm$ 0.08	0.81 $\pm$ 0.11	0.91 $\pm$ 0.11 <sup>**</sup>
	F	0.75 $\pm$ 0.09	0.74 $\pm$ 0.09	0.75 $\pm$ 0.08	0.76 $\pm$ 0.09	0.87 $\pm$ 0.10	0.86 $\pm$ 0.09
Total body (g.cm <sup>-2</sup> )	M	0.88 $\pm$ 0.05	0.89 $\pm$ 0.05	0.89 $\pm$ 0.06	0.90 $\pm$ 0.04	0.91 $\pm$ 0.05	0.97 $\pm$ 0.08 <sup>**</sup>
	F	0.88 $\pm$ 0.10	0.89 $\pm$ 0.06	0.91 $\pm$ 0.06	0.91 $\pm$ 0.06	0.99 $\pm$ 0.06	0.98 $\pm$ 0.06
Volumetric lumbar spine (g.cm <sup>-3</sup> )	M	0.29 $\pm$ 0.03	0.28 $\pm$ 0.03	0.28 $\pm$ 0.03	0.28 $\pm$ 0.03	0.28 $\pm$ 0.02	0.28 $\pm$ 0.03
	F	0.31 $\pm$ 0.02	0.31 $\pm$ 0.03	0.32 $\pm$ 0.02	0.32 $\pm$ 0.03	0.35 $\pm$ 0.03	0.34 $\pm$ 0.03
Volumetric femoral neck (g.cm <sup>-3</sup> )	M	0.43 $\pm$ 0.09	0.45 $\pm$ 0.13	0.41 $\pm$ 0.08	0.42 $\pm$ 0.08	0.40 $\pm$ 0.07	0.46 $\pm$ 0.08
	F	0.48 $\pm$ 0.09	0.43 $\pm$ 0.07	0.41 $\pm$ 0.08	0.44 $\pm$ 0.09	0.49 $\pm$ 0.10	0.47 $\pm$ 0.08

<sup>#</sup> figure in the parentheses are number of subjects

<sup>\*\*</sup> significant difference between level of calcium intake by Mann Whitney U-test in the same sex and pubertal stage at p<0.05

**Table 4.14** Mean  $\pm$  SD of bone mineral content classified by level of calcium intake from FFQ, sex and pubertal stage of subjects.

Bone mineral content	Sex	Pre-puberty		Early-puberty		Late-puberty	
		$\leq 669$ (mg.d <sup>-1</sup> )	$> 669$ (mg.d <sup>-1</sup> )	$\leq 669$ (mg.d <sup>-1</sup> )	$> 669$ (mg.d <sup>-1</sup> )	$\leq 669$ (mg.d <sup>-1</sup> )	$> 669$ (mg.d <sup>-1</sup> )
Midshaft radius (g)	M	0.89 $\pm$ 0.10 (31)	0.94 $\pm$ 0.10 (25)	1.03 $\pm$ 0.14 (35)	1.01 $\pm$ 0.10 (20)	1.14 $\pm$ 0.17 (13)	1.15 $\pm$ 0.07 (12) <sup>#</sup>
	F	0.81 $\pm$ 0.03 (7)	0.88 $\pm$ 0.09** (16)	0.94 $\pm$ 0.13 (49)	0.97 $\pm$ 0.13 (35)	1.15 $\pm$ 0.13 (17)	1.16 $\pm$ 0.21 (13)
Lumbar spine (g)	M	16.7 $\pm$ 2.77	17.7 $\pm$ 3.06	19.9 $\pm$ 4.00	20.5 $\pm$ 2.85	27.8 $\pm$ 6.64	29.0 $\pm$ 4.66
	F	16.6 $\pm$ 2.02	18.7 $\pm$ 2.30**	23.1 $\pm$ 5.35	22.8 $\pm$ 4.27	31.6 $\pm$ 5.95	30.8 $\pm$ 6.31
Femoral neck (g)	M	2.76 $\pm$ 0.51	2.97 $\pm$ 0.96	2.95 $\pm$ 0.50	3.00 $\pm$ 0.40	3.1 $\pm$ 0.95	3.95 $\pm$ 0.49
	F	2.38 $\pm$ 0.46	2.55 $\pm$ 0.40	2.81 $\pm$ 0.56	2.85 $\pm$ 0.50	3.49 $\pm$ 0.46	3.47 $\pm$ 0.61
Total body (g)	M	1131 $\pm$ 134	1174 $\pm$ 160	1348 $\pm$ 258	1364 $\pm$ 139	1656 $\pm$ 257	1796 $\pm$ 238
	F	1041 $\pm$ 77	1187 $\pm$ 129**	1380 $\pm$ 235	1357 $\pm$ 200	1724 $\pm$ 235	1730 $\pm$ 317
Total body calcium (g)	M	430 $\pm$ 51	446 $\pm$ 61	512 $\pm$ 98	518 $\pm$ 53	629 $\pm$ 98	682 $\pm$ 90
	F	396 $\pm$ 29	451 $\pm$ 49**	524 $\pm$ 89	515 $\pm$ 76	655 $\pm$ 89	657 $\pm$ 120

<sup>#</sup> figure in the parentheses are number of subjects

\*\* significant difference between level of calcium intake by Mann Whitney U-test in the same sex and pubertal stage at  $p < 0.05$

bone value at femoral neck BMD ( $p=0.039$ ) and total body BMD ( $p=0.023$ ) at late puberty in male subjects whose dietary calcium intake was greater than mean. Among females, no difference of bone values was observed between groups with different level of calcium intake at any pubertal stage.

#### *Bone mineral content*

Table 4.14 shows the mean  $\pm$  SD of BMC values at different bone sites of the subjects as classified by level of calcium intake. The difference in BMC values among those who had different level of usual dietary calcium intake was found particularly in pre-puberty females who had calcium intake above the group mean. They had significantly greater mean values of BMC at midshaft radius ( $r=0.015$ ), lumbar spine ( $r=0.045$ ), total body BMC ( $r=0.011$ ) and total body calcium ( $p=0.012$ ) than those in low calcium intake group. Among males, there was no significant difference of the BMC values between those with different level of dietary calcium intake at any pubertal stage.

#### **4.3.4 Weight bearing activity**

The average of time spent for weight bearing activity of the 9-12 years old subjects in this study was  $6.5 \pm 5.8$  hr.wk<sup>-1</sup> as shown in Table 4.15. Among subjects with different age groups, no significant difference in the mean value of time spent for weight bearing activity (hr.wk<sup>-1</sup>) was observed in both male and female subjects. However, males significantly spent more time for weight bearing activities than females in all age groups ( $0.000 < p < 0.01$ ).

**Table 4.15** Mean  $\pm$  SD of time spent for weight bearing activities of the subjects according to sex and age group

Variable	Sex	Age group (y)				Total
		9.00-9.99	10.00-10.99	11.00-11.99	12.00-12.99	
Weight bearing activities (hr.wk <sup>-1</sup> )	M	7.9 $\pm$ 6.1 (26)	7.4 $\pm$ 4.9 (35)	8.3 $\pm$ 5.6 (39)	10.6 $\pm$ 8.3 (34)	8.5 $\pm$ 6.4 (137) <sup>#</sup>
	F	4.2 $\pm$ 3.1 * (27)	4.0 $\pm$ 3.1 * (38)	4.5 $\pm$ 5.6 * (37)	4.9 $\pm$ 4.6 * (35)	4.4 $\pm$ 4.3 * (137)
	Total	6.1 $\pm$ 5.2 (56)	5.6 $\pm$ 4.4 (73)	6.4 $\pm$ 5.9 (76)	7.7 $\pm$ 7.3 (69)	6.5 $\pm$ 5.8 (274)

# figures in the parentheses are number of subjects

\* significant difference between sex at  $p < 0.05$

**Table 4.16** Mean  $\pm$  SD of bone mineral density classified by sex and level of activity of subjects

Bone mineral density	Sex	Level of weight bearing activity	
		$\leq 6.5$ hr.wk <sup>-1</sup>	$>6.5$ hr.wk <sup>-1</sup>
Midshaft radius (g.cm <sup>-2</sup> )	M	0.48 $\pm$ 0.05 (62)	0.50 $\pm$ 0.06 (75) <sup>#</sup>
	F	0.51 $\pm$ 0.06 (104)	0.50 $\pm$ 0.06 (33)
Lumbar spine (g.cm <sup>-2</sup> )	M	0.72 $\pm$ 0.07	0.76 $\pm$ 0.09***
	F	0.84 $\pm$ 0.12	0.85 $\pm$ 0.08
Femoral neck (g.cm <sup>-2</sup> )	M	0.76 $\pm$ 0.09	0.81 $\pm$ 0.11***
	F	0.78 $\pm$ 0.10	0.78 $\pm$ 0.09
Total body (g.cm <sup>-2</sup> )	M	0.88 $\pm$ 0.05	0.91 $\pm$ 0.06***
	F	0.92 $\pm$ 0.07	0.92 $\pm$ 0.06
Volumetric lumbar spine (g.cm <sup>-3</sup> )	M	0.28 $\pm$ 0.03	0.28 $\pm$ 0.03
	F	0.32 $\pm$ 0.03	0.33 $\pm$ 0.02
Volumetric femoral neck (g.cm <sup>-3</sup> )	M	0.41 $\pm$ 0.09	0.43 $\pm$ 0.09
	F	0.44 $\pm$ 0.09	0.43 $\pm$ 0.07

<sup>#</sup> figure in the parentheses are number of subjects.

\*\*\* significant difference between level of activity at  $p < 0.005$

**Table 4.17** Mean  $\pm$  SD of bone mineral content classified by level of activity and sex of subjects

Bone mineral content	Sex	Level of weight bearing activity	
		$\leq 6.5 \text{ hr.wk}^{-1}$	$> 6.5 \text{ hr.wk}^{-1}$
Midshaft radius (g)	M	0.97 $\pm$ 0.13 (62)	1.02 $\pm$ 0.15 (75) <sup>#</sup>
	F	0.98 $\pm$ 0.16 (103)	0.99 $\pm$ 0.18 (33)
Lumbar spine (g)	M	19.2 $\pm$ 4.3	21.4 $\pm$ 6.2*
	F	23.8 $\pm$ 6.8	24.0 $\pm$ 5.2
Femoral neck (g)	M	2.91 $\pm$ 0.67	3.20 $\pm$ 0.75*
	F	2.93 $\pm$ 0.60	2.88 $\pm$ 0.61
Total body BMC (g)	M	1275 $\pm$ 224	1386 $\pm$ 321*
	F	1405 $\pm$ 296	1427 $\pm$ 263
Total body calcium (g)	M	485 $\pm$ 85	527 $\pm$ 122*
	F	534 $\pm$ 112	542 $\pm$ 100

<sup>#</sup> figure in the parentheses are number of subjects.

\* significant difference between level of activity at  $P < 0.05$

We hypothesized that duration of weight bearing activity as determined by the number of hours spent for weight bearing activity on the weekly basis might have greater impact on bone gain in children. Therefore, we classified the subjects in each sex into 2 groups; 1. Less active group ( $\leq 6.5$  hr.wk<sup>-1</sup>), 2. Active group ( $> 6.5$  hr.wk<sup>-1</sup>) by using the group mean value of time spent for weight bearing activity as a cut off point.

#### *Bone mineral density*

It was found that active male subjects who had the number of time spent for weight bearing activities of more than 6.5 hr.wk<sup>-1</sup> had significantly greater BMD value than those who was less active at lumbar spine ( $p=0.004$ ), femoral neck ( $p=0.007$ ) total body BMD ( $p=0.02$ ) except for the value at midshaft radius ( $p=0.058$ ) as shown in Table 4.16.

#### *Bone mineral content*

For BMC, it was found that the active males who spent time for weight bearing activities of more than 6.5 hr.wk<sup>-1</sup> had significantly greater BMC than the less active group at lumbar spine ( $p=0.022$ ), femoral neck ( $p=0.021$ ), total body BMC ( $p=0.019$ ) and total body calcium ( $p=0.019$ ) except for the value at midshaft radius ( $p=0.058$ ).

The results revealed no significant difference in bone mass values between less active and active female subjects.

#### 4.4 Relationship between different variables on bone parameters

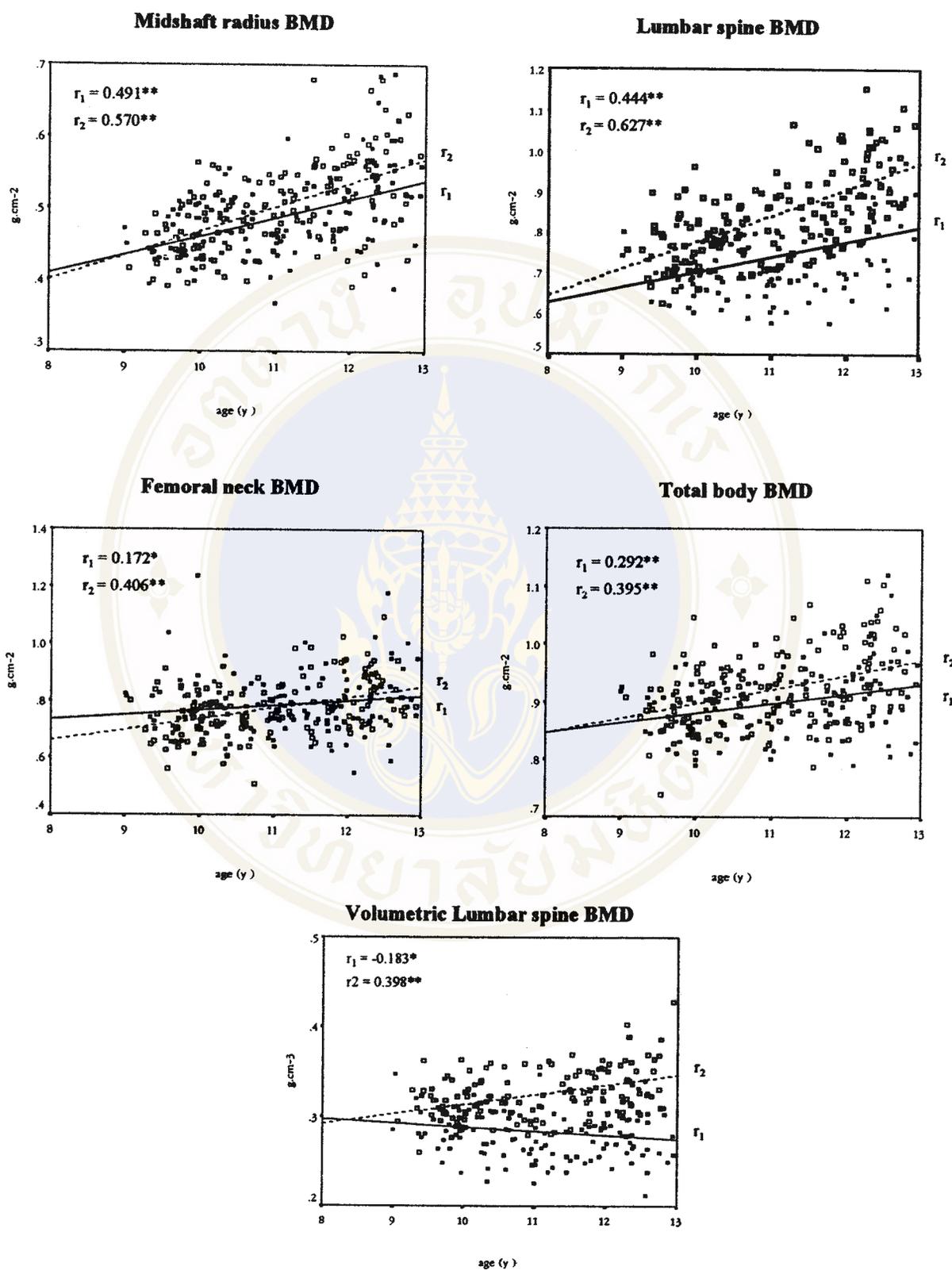
##### 4.4.1 Age

###### *Bone mineral density*

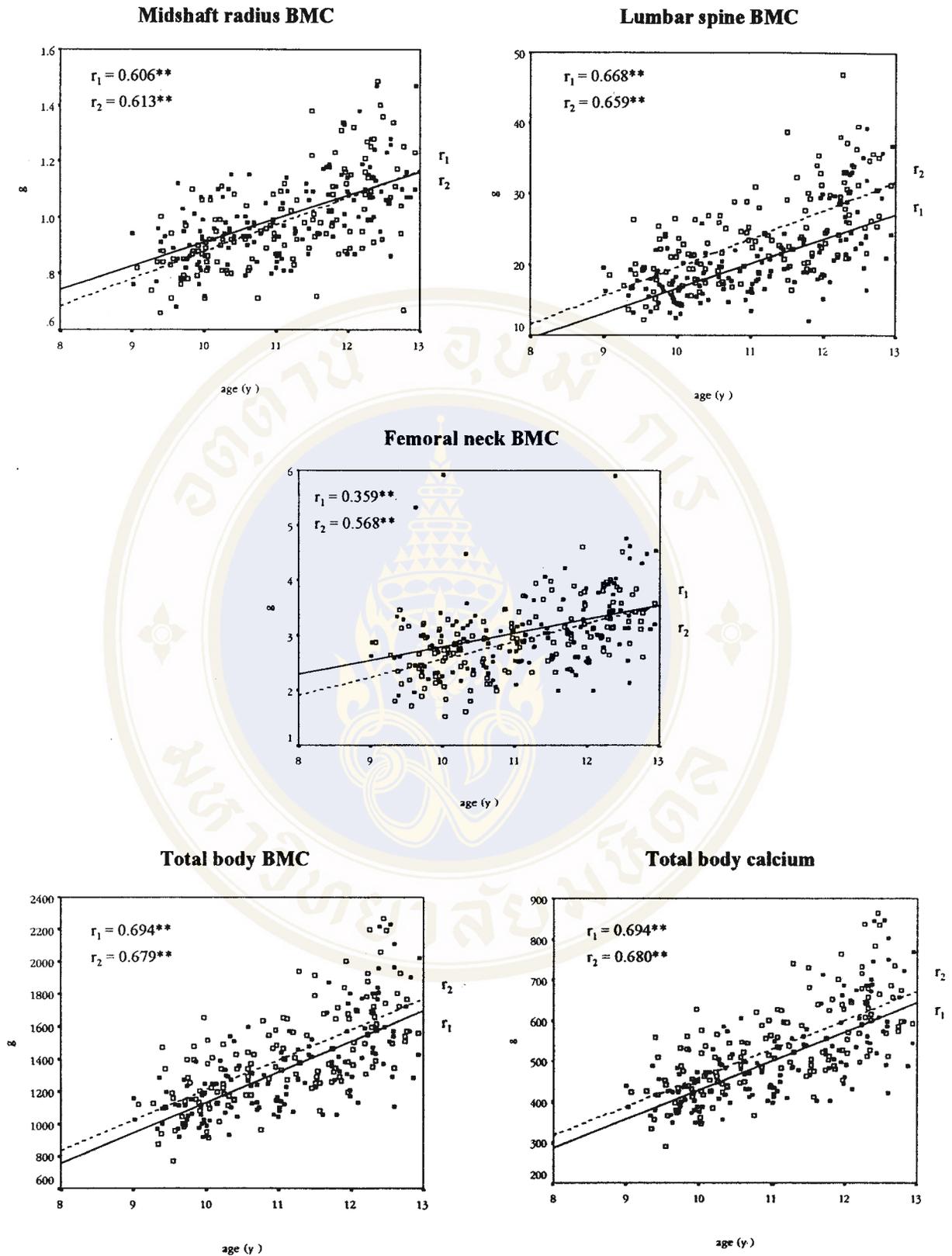
Figure. 5 demonstrates the relationship between age and BMD of the study subjects. Among males, the correlation coefficient showed moderate positive correlation between age and BMD at midshaft radius ( $r=0.491$ ,  $p<0.01$ ), lumbar spine ( $r=0.444$ ,  $p<0.01$ ) femoral neck ( $r=0.172$ ,  $p<0.05$ ) and total body ( $r=0.292$ ,  $p<0.01$ ). Among females, the positive correlation between age and BMD was found as well at midshaft radius ( $r=0.570$ ,  $p<0.01$ ), lumbar spine ( $r=0.627$ ,  $p<0.01$ ) femoral neck ( $r=0.406$ ,  $p<0.01$ ) and total body ( $r=0.395$ ,  $p<0.01$ ). Interestingly, there was a significant negative correlation between age and volumetric BMD at lumbar spine ( $r=-0.183$ ,  $p<0.05$ ) in males. While significant positive correlation was shown in females ( $r=0.398$ ,  $p<0.01$ ). No significant correlation between age and volumetric BMD at femoral neck was observed for females.

###### *Bone mineral content*

Age also had significant correlation with BMC in both males and females as shown in Figure 6. Among males, the correlation coefficient showed positive correlation between age and BMC at midshaft radius ( $r=0.606$ ,  $p<0.01$ ), lumbar spine ( $r=0.668$ ,  $p<0.01$ ), femoral neck ( $r=0.359$ ,  $p<0.01$ ), total body BMC ( $r=0.694$ ,  $p<0.01$ ) and total body calcium ( $r=0.694$ ,  $p<0.01$ ). Similar result were found in females as following : at midshaft of radius ( $r=0.613$ ,  $p<0.01$ ), lumbar spine ( $r=0.659$ ,  $p<0.01$ ), femoral neck ( $r=0.568$ ,  $p<0.01$ ), total body BMC ( $r=0.679$ ,  $p<0.01$ ) and total body calcium ( $r=0.680$ ,  $p<0.01$ ).



**Figure 5** Relationship between age and bone mineral density (g.cm<sup>-2</sup>) at midshaft radius, lumbar spine, femoral neck and total body of the subjects ;  
 $r_1$  (—),  $r_2$  (---) correlation coefficient of males and females respectively;  
 \*, \*\* significant at  $p < 0.05$ ,  $p < 0.01$  respectively. Each dot correspond one individual.



**Figure 6** Relationship between age and bone mineral content (g) at midshaft radius, lumbar spine, femoral neck, total body BMC and total body calcium of the subjects ;  $r_1$  (—),  $r_2$  ( - - ) correlation coefficient of males and females respectively; \*, \*\* significant at  $p < 0.05$ ,  $p < 0.01$  respectively. Each dot correspond one individual.

#### 4.4.2 Weight and Height

To determine the relationship between weight, height and bone values, an approach of weight for height were used because we found the collinearity between weight for age, height for age and weight for height

##### *Bone mineral density*

It was found that weight for height had a positive correlation with BMD for both males and females as displayed in Table 4.18. Among males, the correlation coefficient exhibited a significant correlation between weight for height and BMD at midshaft radius ( $r=0.460$ ,  $p<0.01$ ), lumbar spine ( $r=0.580$ ,  $p<0.01$ ), femoral neck ( $r=0.372$ ,  $p<0.01$ ) and total body ( $r=0.521$ ,  $p<0.01$ ). Similar correlations were found as well in females at midshaft radius ( $r=0.548$ ,  $p<0.01$ ), lumbar spine ( $r=0.644$ ,  $p<0.01$ ), femoral neck ( $r=0.552$ ,  $p<0.01$ ) and total body ( $r=0.615$ ,  $p<0.01$ ). There was no significant correlation between weight for height and volumetric BMD in both males and females.

##### *Bone mineral content*

Weight for height had positive correlation with BMC for both males and females as displayed in Table 4.19. Among males, the significant correlation was found at midshaft radius ( $r=0.681$ ,  $p<0.01$ ), lumbar spine ( $r=0.735$ ,  $p<0.01$ ), femoral neck ( $r=0.432$ ,  $p<0.01$ ), total body BMC ( $r=0.800$ ,  $p<0.01$ ), and total body calcium ( $r=0.800$ ,  $p<0.01$ ). Similar results were found in females as well at midshaft radius ( $r=0.612$ ,  $p<0.01$ ), lumbar spine ( $r=0.710$ ,  $p<0.01$ ), femoral neck ( $r=0.646$ ,  $p<0.01$ ), total body BMC ( $r=0.817$ ,  $p<0.01$ ) and total body calcium ( $r=0.818$ ,  $p<0.01$ ).

#### 4.4.3 Dietary calcium intake

Figure 7 demonstrates the relationship between dietary calcium intake and bone values for males and females. Among males, there were significant correlations between dietary calcium intake and BMD at midshaft radius ( $r=0.199$ ,  $p<0.05$ ) and BMC at femoral neck ( $r=0.178$ ,  $p<0.05$ ). The significant correlation was not observed between dietary calcium and volumetric BMD among males. Among females, significant positive correlation was only found between dietary calcium intake and BMC at midshaft radius ( $r=0.207$ ,  $p<0.05$ ). The significant correlation was not observed between dietary calcium and areal BMD or volumetric BMD values in females.

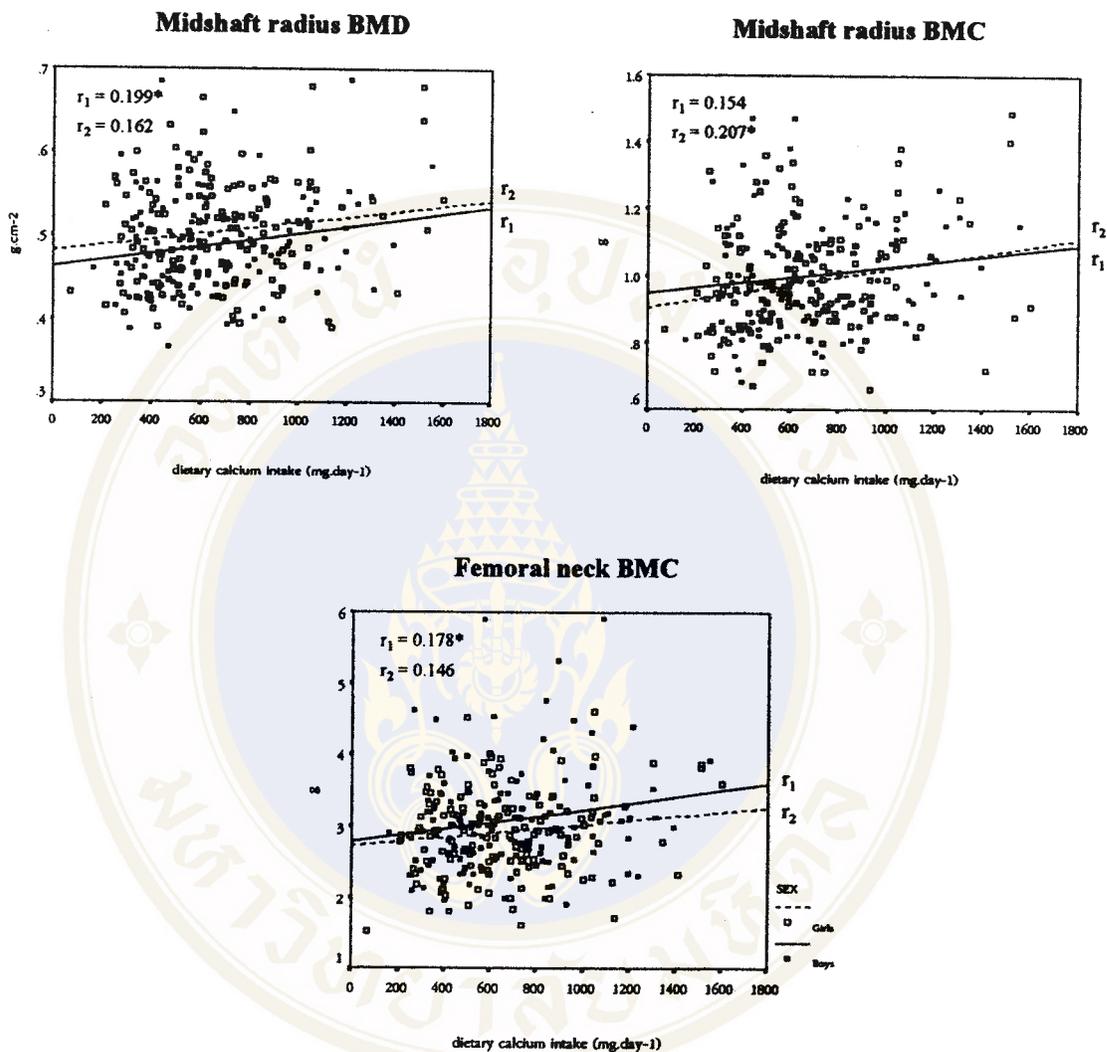
#### **4.4.4 Weight bearing activity**

##### *Bone mineral density*

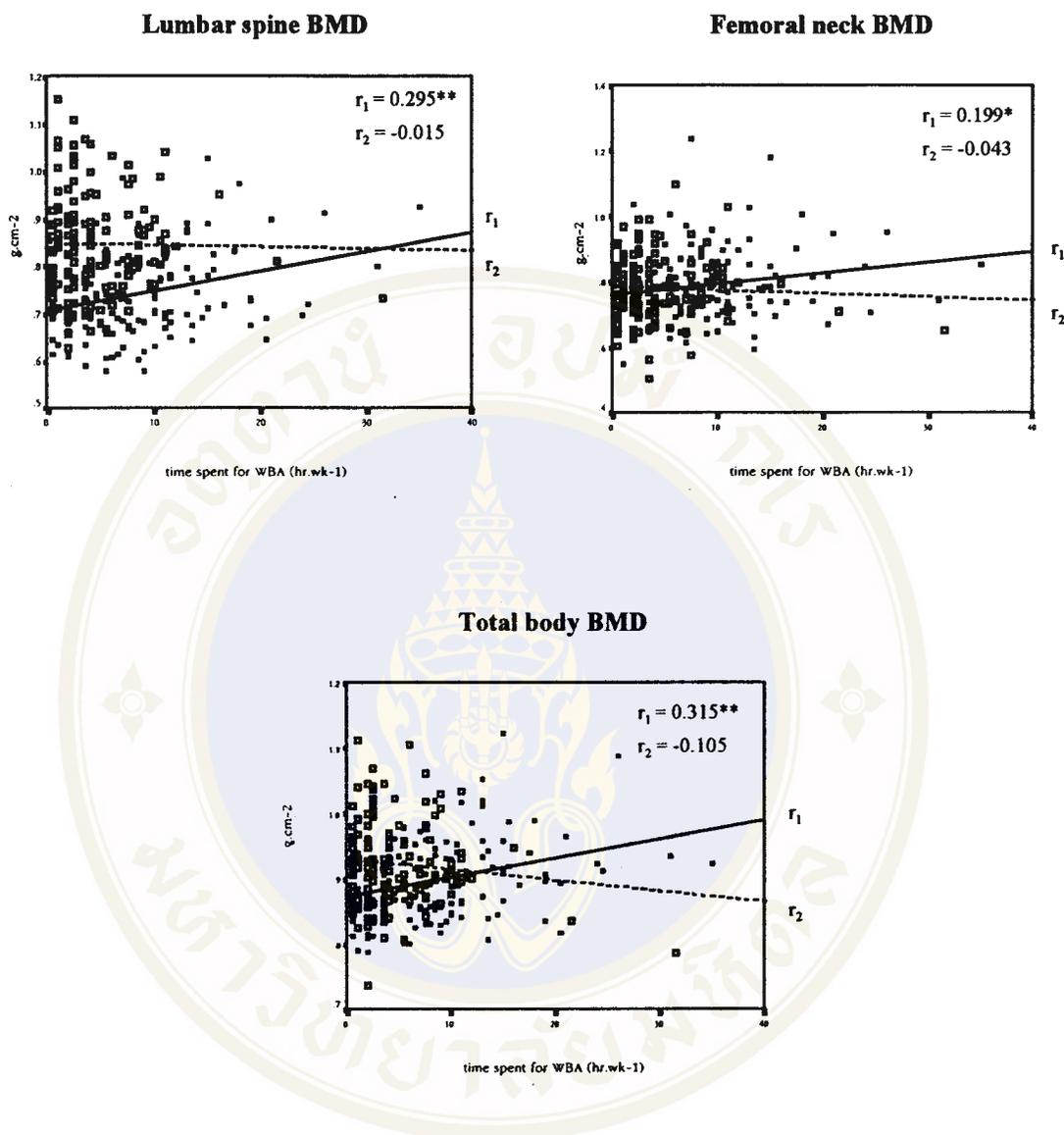
The relationship between weight bearing activity and BMD at various bone sites are shown in Figure 8. Among males, there were positive correlation between weight bearing activities and BMD at lumbar spine ( $r=0.295$ ,  $p<0.01$ ), femoral neck ( $r=0.199$ ,  $p<0.05$ ) and total body ( $r=0.315$ ,  $p<0.01$ ).

##### *Bone mineral content*

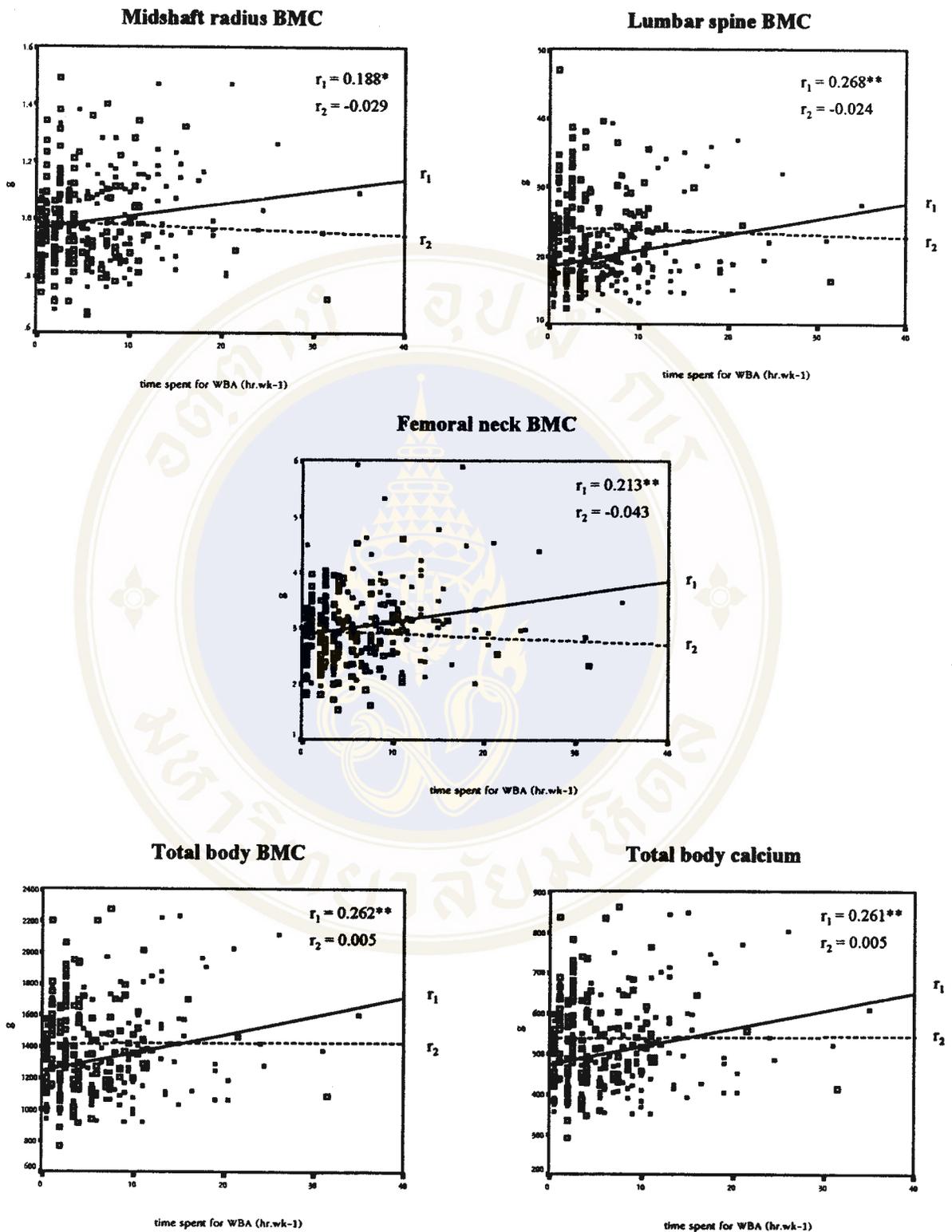
Males also had significant positive correlation between weight bearing activities and BMC at midshaft radius ( $r=0.188$ ,  $p<0.05$ ), lumbar spine ( $r=0.268$ ,  $p<0.01$ ), femoral neck ( $r=0.213$ ,  $p<0.01$ ) total body BMC ( $r=0.262$ ,  $p<0.01$ ) and total body calcium ( $r=0.261$ ,  $p<0.01$ ) as shown in figure 9. The significant correlation was not observed between weight bearing activity and bone values among females. All correlation coefficient of age, weight for height, usual dietary calcium intake, time spent for weight bearing activity were summarized in Table 4.18-4.19.



**Figure 7** Relationship between dietary calcium intake and bone mineral density ( $g.cm^{-2}$ ) at midshaft radius and bone mineral content (g) at midshaft radius and femoral neck of the subjects ;  $r_1$  (—),  $r_2$  (- - -) correlation coefficient of males and females respectively ; \*, \*\* significant at  $p < 0.05$ ,  $p < 0.01$  respectively Each dot correspond one individual.



**Figure 8** Relationship between weight bearing activity and bone mineral density(g.cm<sup>-2</sup>) at lumbar spine, femoral neck and total body of the subjects ;  
 $r_1$  (—),  $r_2$  (- - -) correlation coefficient of males and females respectively;  
 \*, \*\* significant at  $p < 0.05$ ,  $p < 0.01$  respectively  
 Each dot correspond one individual.



**Figure 9** Relationship between weight bearing activity and bone mineral content (g) at midshaft radius, lumbar spine, femoral neck, total body BMC and total body calcium of the subjects ;

$r_1$  (—),  $r_2$  ( - - ) correlation coefficient of males and females respectively;

\*, \*\* significant at  $p < 0.05$ ,  $p < 0.01$  respectively

Each dot correspond one individual.

**Table 4.18** Correlation coefficient between various factors and bone mineral density of the subjects

Bone mineral density	Sex	Age	Weight for height	Dietary calcium intake	Time spent for WBA
Midshaft radius (g.cm <sup>-2</sup> )	M	0.491**	0.460**	0.199*	0.167
	F	0.570**	0.548**	0.162	-0.103
Lumbar spine (g.cm <sup>-2</sup> )	M	0.444**	0.580**	0.060	0.295**
	F	0.627**	0.644**	0.118	-0.015
Femoral neck (g.cm <sup>-2</sup> )	M	0.172*	0.372**	0.163	0.199*
	F	0.406**	0.552**	0.119	-0.043
Total body (g.cm <sup>-2</sup> )	M	0.292**	0.521**	0.165	0.315**
	F	0.395**	0.615**	0.125	-0.105
Volumetric Lumbar spine (g.cm <sup>-3</sup> )	M	-0.183*	-0.047	-0.118	0.041
	F	0.398**	0.167	0.096	-0.022
Volumetric femoral neck (g.cm <sup>-3</sup> )	M	-0.151	-0.038	0.014	0.077
	F	0.068	0.168	0.062	-0.098

\*, \*\* significant difference between sex at p<0.05, p<0.01 respectively

WBA : weight bearing activities

**Table 4.19** Correlation coefficient between various factors and bone mineral content of the subjects

Bone mineral content	Sex	Age	Weight for height	Dietary calcium intake	Time spent for WBA
Midshaft radius (g)	M	0.606**	0.681**	0.154	0.188*
	F	0.613**	0.612**	0.207*	-0.029
Lumbar spine (g)	M	0.668**	0.735**	0.111	0.268**
	F	0.659**	0.710**	0.100	-0.024
Femoral neck (g)	M	0.359**	0.432**	0.178*	0.213*
	F	0.568**	0.646**	0.146	-0.043
Total body (g)	M	0.694**	0.800**	0.157	0.262**
	F	0.679**	0.817**	0.135	-0.005
Total body calcium (g)	M	0.694**	0.800**	0.157	0.261**
	F	0.680**	0.818**	0.135	-0.005

\*, \*\* significant difference between sex at  $p < 0.05$ ,  $p < 0.01$  respectively

WBA : weight bearing activities



#### 4.5 Multiple regression analysis

Multiple regression analysis was used to investigate the influence of physiological factors and environmental factors. During performing the multiple regression analysis, we conducted various regression models to determine independent and combined effects of physiological and environmental factors. In the first model, we entered the physiological factors (age, sex, weight for height and pubertal status) as independent variables. The second model comprised of environmental factors (usual calcium intake and number of hour spent for weight bearing activity) as independent variables. The final model, all physiological and environmental factors were entered simultaneously as independent variables. All models were used by considering each bone parameter as dependent variable. A set of predictive variables of each value of bone parameters was selected by using the enter method.

Most of variables were continuous variables except for sex and pubertal stage, which were coded as dummy variables as follows; sex (males=0, females=1), pubertal stage pre-puberty (= 1), versus maturity (= 0), and early-puberty (= 1) versus maturity (= 0). All final models of the significant predictive variables of each bone parameter and the values of adjusted  $R^2$  of each predictive model were summarized in Table 4.20-4.21.

##### *Bone mineral density*

Result from multiple regression analysis in the final model showed that age, weight for height, pre-puberty, early-puberty and usual calcium intake were the significant determinants of the BMD at midshaft radius which accounting for 40% of the variation of the BMD value (adjusted  $R^2 = 0.402$ ) as age was the most significant

predictor (BETA = 0.311). For lumbar spine BMD; age, sex, weight for height, pre-puberty, early puberty and weight bearing activity were the significant predictors which describe 61% of variability (adjusted  $R^2 = 0.613$ ). Sex was the most significant of all variable (BETA = 0.482). For femoral neck BMD; weight for height, pre-puberty and early puberty were the significant variables, accounting for 23% of the variability as weight for height was the most significant predictor (BETA = 0.377). Sex, weight for height, pre-puberty and early-puberty were the significant predictors affecting on total body BMD, accounting for 37% of total variance (adjusted  $R^2 = 0.371$ ); weight for height was the most significant predictor (BETA = 0.476).

Sex, pre-puberty and early-puberty were the significant predictors on volumetric BMD at lumbar spine, responsible for 39% of total variation (adjusted  $R^2 = 0.385$ ). Sex was the most significant predictor (BETA = 0.611). Pre-puberty and early-puberty were the variables that could predict the value of femoral neck volumetric BMD.

In summary, among physiological factors, weight for height and pubertal status were identified as the major determinants for areal BMD for total body as well as at each bone site. As weight increased, we expected the positive change of BMD value, In addition, as children approach puberty period, the rate of bone gain also increase as compare to the pre-pubertal period. Result from multiple regression analysis also demonstrated that among our study population, females had higher total BMD and at lumbar spine than males. Usual calcium intake could positive determine the variation of BMD value at midshaft radius. Time spent for weight bearing activity was the variable which could significantly describe the variation of total body BMC as well as total body calcium.

**Table 4.20** Multiple regression analysis of various factors and bone mineral density

Bone mineral density	b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equation
<b>Midshaft radius (g.cm<sup>-2</sup>)</b>						
Age	0.018	0.004	0.311	0.000	0.402	0.05
Weight for height	0.330	0.082	0.232	0.000		
Pre-puberty	-0.031	0.012	-0.239	0.007		
Early-puberty	-0.030	0.008	-0.252	0.000		
Usual calcium intake	0.000028	0.000	0.136	0.005		
Constant	0.220	0.047	-	0.000		
<b>Lumbar spine (g.cm<sup>-2</sup>)</b>						
Age	0.0198	0.006	0.185	0.001	0.612	0.07
Sex	0.108	0.010	0.482	0.000		
Weight for height	0.854	0.124	0.319	0.000		
Pre-puberty	-0.072	0.017	-0.292	0.000		
Early-puberty	-0.075	0.013	-0.335	0.000		
Weight bearing activity	0.0016	0.001	0.085	0.045		
Constant	0.351	0.071	-	0.000		
<b>Femoral neck (g.cm<sup>-2</sup>)</b>						
Weight for height	0.922	0.153	0.377	0.000	0.241	0.09
Pre-puberty	-0.040	0.019	-0.174	0.036		
Early-puberty	-0.058	0.016	-0.285	0.000		
Constant	0.587	0.047	-	0.000		
<b>Total body (g.cm<sup>-2</sup>)</b>						
Sex	0.023	0.006	0.183	0.000	0.371	0.05
Weight for height	0.731	0.088	0.476	0.000		
Pre-puberty	-0.026	0.011	-0.186	0.015		
Early-puberty	-0.032	0.009	-0.248	0.000		
Constant	0.712	0.029	-	0.000		
<b>Volumetric lumbar spine (g.cm<sup>-3</sup>)</b>						
Sex	0.043	0.004	0.611	0.000	0.385	0.03
Pre-puberty	-0.011	0.005	-0.145	0.026		
Early-puberty	-0.015	0.004	-0.208	0.001		
Constant	0.250	0.007	-	0.000		
<b>Volumetric femoral neck (g.cm<sup>-3</sup>)</b>						
Pre-puberty	-0.016	-0.015	-0.081	0.306	0.026	0.09
Early-puberty	-0.039	0.014	-0.223	0.005		
Constant	0.457	0.012	-	0.000		

*Bone mineral content*

Age, sex, weight for height, pre-puberty, early-puberty and usual calcium intake were identified as significant variables which was responsible for 55% of the total variability of midshaft radius BMC (adjusted  $R^2 = 0.546$ ). For lumbar spine, all physiological factors were the major determinant which accounting for 71% of total variation (adjusted  $R^2 = 0.713$ ). For femoral neck; age, sex, weight for height, pre-puberty and early-puberty were the significant predictors and accounted for 37% of the variability (adjusted  $R^2 = 0.367$ ). For total body BMC and total body calcium, all physiological factors and weight bearing activity were the significant predictors accounting for 79% of total variability (adjusted  $R^2 = 0.790$ ).

Common variables which accounted for the variability on BMC value were age, sex, weight for height and puberty. Usual calcium intake was the environmental factor which could significantly describe the variation on BMC at midshaft radius whereas weight bearing activity was the variable which predicted the bone value at total body BMC and total body calcium.

**Table 4.21** Multiple regression analysis of various factors and bone mineral contents

Bone mineral content	b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equatio
<b>Midshaft radius (g)</b>						
Age	0.048	0.008	0.322	0.000	0.546	0.10
Sex	-0.029	0.014	-0.094	0.042		
Weight for height	1.411	0.187	0.380	0.000		
Pre-puberty	-0.072	0.026	-0.211	0.006		
Early-puberty	-0.059	0.019	-0.189	0.003		
Usual calcium intake	0.000066	0.000	0.123	0.004		
Constant	0.126	0.107	-	0.238		
<b>Lumbar spine (g)</b>						
Age	1.591	0.272	0.271	0.000	0.713	3.35
Sex	3.463	0.462	0.278	0.000		
Weight for height	61.718	6.025	0.413	0.000		
Pre-puberty	-4.599	0.849	-0.375	0.000		
Early-puberty	-4.287	0.621	-0.344	0.000		
Constant	-10.082	3.417	-	0.003		
<b>Femoral neck (g)</b>						
Age	0.115	0.043	0.179	0.008	0.367	0.53
Sex	-0.144	0.073	-0.107	0.051		
Weight for height	5.102	0.962	0.317	0.000		
Pre-puberty	-0.352	0.134	-0.236	0.009		
Early-puberty	-0.392	0.098	-0.292	0.000		
Constant	0.626	0.546	-	0.253		
<b>Total body BMC (g)</b>						
Age	76.8	10.7	0.279	0.000	0.789	132.5
Sex	63.0	18.1	0.109	0.001		
Weight for height	375.9	236	0.545	0.000		
Pre-puberty	-166.8	33.2	-0.262	0.000		
Early-puberty	-132.0	24.3	-0.229	0.000		
Weight bearing activity	3.176	1.54	0.064	0.040		
Constant	-405.6	134.9	-	0.003		
<b>Total body calcium (g)</b>						
Age	29.1	4.063	0.279	0.000	0.790	50.32
Sex	23.9	6.87	0.109	0.001		
Weight for height	1428.4	89.7	0.545	0.000		
Pre-puberty	-63.3	12.6	-0.262	0.000		
Early-puberty	-50.1	9.24	-0.229	0.000		
Weight bearing activity	1.20	0.584	0.064	0.040		
Constant	-154.12	51.2	-	0.003		

## CHAPTER V

### DISCUSSION

Several studies reported that factors affecting bone mass in children and adolescent were age, sex, pubertal stage, weight, height, dietary calcium intake and physical activity.<sup>(10,57,59,71,72,114)</sup> All factors determined in this study were categorized into 2 groups; physiological factors (age, sex, weight, height and pubertal stage) and environmental factors (dietary calcium intake, weight bearing activity). The result showed that the major determinants were physiological factors which accounted for the variability whereas environmental factors were the minor determinants of bone mass.

#### **Age, gender and puberty**

It was clear that puberty had a major effect on bone mass gain in children and adolescents. Age, gender and pubertal development were closely correlated.<sup>(115,116)</sup> As age increased, children approached to pubertal period. Bone mass was markedly increased in advancing age and pubertal stage. Like previous studies,<sup>(60,72,117)</sup> subjects who were in early- and late-pubertal stage had significant greater bone mineral content than those in prepubertal stage (Table 4.11). Matkovic stated that bone mass was obviously accumulated between pubertal stage 2 and 4.<sup>(36)</sup> In this study, bone mass was significantly greater at the age of 11 to 12 years, coincided with pubertal development,

as age onset of puberty started at 10 years for females and 11 years for males (Table 4.1). During puberty, there was interaction between growth hormone, sex steroid hormone and insulin like growth factor-I which promote skeletal growth.<sup>(36)</sup>

Not only bone mass but also bone size increased dramatically during puberty.<sup>(54)</sup> Consequently, BMD increased in late puberty (Table 4.12).

Bone mass seem to be higher in female compared with male subjects because of the early onset of puberty in female especially in lumbar spine. It was confirmed by Slemenda et al. who reported that skeletal mineralization accelerated markedly in spine, and less markedly in femoral neck and radius at puberty in the three year observational study in 90 children aged 6-14 years.<sup>(91)</sup> So the total body BMD was also higher in female because lumbar spine had more contribution of bone mass than other sites. Males had higher bone mass than females in younger children in this study in contrast with previous reports.<sup>(10,57,118)</sup> It may be partly due to the average time spent for weight bearing activity in male subjects which was greater than 7 hours.wk<sup>-1</sup>, and higher than females in all age groups (Table 4.15). Some studies found that children who spent time for weight bearing activity for more than 7 hours.wk<sup>-1</sup> had greater bone mass than those who took less time especially in femoral neck.<sup>(25,88)</sup> However, several studies reported that males will have higher bone mass value than females when their sexual development reached the maturation in later age.<sup>(37,59)</sup>

In this study, volumetric BMD significantly decreased at femoral neck at early pubertal stage (Table 4.11 and Figure 4). This may be partly due to the increase of bone mineral content not in proportion to the increase in the enlarging bone. Among male subjects, the BMC value from pre- to early-puberty at femoral neck increased

3.9% (2.85 g.cm<sup>-2</sup> to 2.96 g.cm<sup>-2</sup>) whereas the increase in bone volume was 11% (6.61 cm<sup>3</sup> to 7.32 cm<sup>3</sup>). Since volumetric BMD was calculated from BMC and bone volume, this disproportional increase in bone mass and bone volume resulted in decreased volumetric BMD. The same finding was also demonstrated in females but the reduction of volumetric BMD at this site did not reach statistical significance.

### **Weight and Height**

Many studies reported that body weight was a major predictor of BMD in premenopausal women<sup>(119,120)</sup> whereas height was found to be the best predictor of bone mass in children and adolescents.<sup>(121)</sup> During the rapid bone growth, bone mass greatly changes especially in long bone. To determine the effect of growth parameter on bone mass in children, an approach of weight for height was used to normalize the degree of mineralization. This result revealed that weight for height was the most important predictor on bone mass in Thai children and adolescents. It indicated that children should have an appropriate weight compared to height to maintain the loading on bone which stimulated bone formation. Weight was associated with sexual development which in turn affect bone mass. The study by Stark et al. found that girls with early menarche were more likely to be over weight<sup>(122)</sup> and after adjusted for body weight, puberty was found to be the most significant predictor of bone mass.<sup>(117)</sup> McCormick stated that spinal mineralization increased with body weight, however, vertebral volume was apparently a limit factor.<sup>(123)</sup> As weight continued to increase beyond 95<sup>th</sup> percentile, further increase in mineralization were restricted. Furthermore, Mazoni et al. reported that there was no difference in bone mineral content, either total

or regional BMC, between obese and normal weight children after correction for age and sex in children aged 5-18 years.<sup>(124)</sup> Conversely, some females who try to control or lose their weight for beauty image might have an adverse effect on bone mass due to an inappropriate weight for height.<sup>(125)</sup> There was report which demonstrated that women with anorexia nervosa, an eating disorder, had low bone mass.<sup>(126)</sup>

### **Dietary calcium intake**

Dietary assessment in this study showed that current dietary calcium intake of Thai children was  $511 \pm 230$  mg.d<sup>-1</sup> (Table 4.3). It was quite close to Hong Kong Chinese Children whose intake were 570 mg.d<sup>-1</sup>.<sup>(79)</sup> Their consumption were lower than Caucasian children whose calcium intake were greater than 1200 mg.d<sup>-1</sup>.<sup>(60,72)</sup> Furthermore, Thai children had inadequate calcium intake on the basis of the current Thai RDA in this age group (800-1200 mg.d<sup>-1</sup>). Only 10% of total subjects had reached these recommendation (Table 4.6).

According to the validation study, the usual calcium intake from sFFQ was able to estimate group mean intake and correctly classified children into quartile according to their calcium intake level. In this study, the mean usual calcium intake was  $669 \pm 291$  mg.d<sup>-1</sup> (Table 4.8). There was no significant difference of usual calcium intake between male and female subjects. When compared with actual calcium intake from 3-day food record, the usual calcium intake had higher mean value. However, the mean calcium intake from sFFQ was still under the RDA recommendation. The major sources of usual calcium intake derived from milk and milk products which contributed

57% of total calcium intake (Table 4.8). The second source was fish and aquatic animals (12%) followed by snack and dessert (6%), cereal and grain (5%) and vegetable (5%).

According to Piaseu study, which was conducted in Thai adults, the mean calcium intake was  $361 \text{ mg.d}^{-1}$ .<sup>(127)</sup> The major sources of calcium was also milk and milk product but the contribution was only 18% of total calcium intake. The other sources of calcium were fish 16.3%, vegetable (15.6%), cereal (14.8%), legume (9.6%) and fruit (9.1%). The ratio of calcium from milk : other sources in different calcium levels were 0.9:1, 1:1, 1.7:1, 1.6:1, 1.9:1, 1.7:1 in <400, 401-600, 601-800, 801-1000, 1001-1200, >1200 mg, respectively. It seemed likely that the higher dietary calcium intake, the more milk and milk products were consumed. Lee et al. reported that the mean usual calcium intake of 5 year old children in Hong Kong was  $546 \pm 325 \text{ mg.d}^{-1}$ .<sup>(128)</sup> Milk was the major source of calcium which contributed 43% of total calcium intake. Other sources were cereal (16.6%), vegetable (12.5%), fish (6.7%), fruit (6.1%) and bean (1.9%).

Sandler et al found that the effect of milk consumption in childhood and adolescent on BMD manifest as higher bone density in menopause by the augmentation of peak bone mass.<sup>(129)</sup> Teegarden also found that adolescent milk intake predicted BMD and BMC at midshaft radius, total body BMD and total body BMC in 18-31 year old women after weight was controlled.<sup>(130)</sup> Therefore increasing milk intake during early childhood and adolescence will help forming the favorable nutritional habit leading to maximizing peak bone mass and adequate calcium intake until adult.

Cadogan et al conducted the milk supplementation of 568 ml of whole or reduced fat milk per day in 82 white girls aged 12.2 years for 18 months.<sup>(131)</sup> They found that

the supplemented group had greater increased BMD value and serum concentrations of insulin like growth factor I (IGF-I) than control group. The result of this trial indicated that increased milk consumption in adolescence resulted in greater total skeletal mineral acquisition and could modify peak bone mass through IGF-I by enhancing chondrocyte proliferation in the growth plate and matrix formation.

In the promotion of milk intake, some may have problem such as lactose intolerance. Therefore, the source of calcium should derived from other food such as fish and dark green leafy vegetables (kale, broccoli). Recent study found that many dark green leafy vegetables were known to have relatively high calcium and have been presumed to be good source of calcium because they had the same bioavailability as milk.<sup>(132,133)</sup> The dark green leafy vegetable seems to be a suitable choice to maintain adequate calcium intake for some children who may not be willing or unable to obtain as much calcium as they need from dairy source. Interestingly, the children had higher sodium intake than the RDI recommendation (Table 4.3 and 4.4) which might have an adverse effect on bone mass gain.<sup>(85)</sup>

This study demonstrated the relationship between calcium and bone mass in this age group. Male subjects in late puberty receiving calcium intake more than mean had higher BMD at femoral neck and total body BMD than the children receiving less than mean. For females subjects, pre-puberty children whose calcium intake was more than mean had higher BMC at midshaft radius, lumbar spine, total body BMC, total body calcium than children whose intake was less than mean. Result from multiple regression analysis demonstrated that usual calcium intake was a significant predictor of BMD and BMC at midshaft radius. From studies on the effect of calcium supplement

on BMD in young twins given calcium or placebo<sup>(8)</sup> and Thai children aged 9-12 years<sup>(134)</sup> reported similar result. In addition, the studies also revealed that supplemented group had greater BMD at midshaft radius than placebo group during pre-puberty stage. The question arises as why calcium supplementation during pre-puberty stage had an effect only on midshaft radius. Seeman et al. suggested that bone growth during the rapid growth period usually occurs in long bone through the process of periosteal growth for 70% and endosteal growth for 30%.<sup>(54)</sup> Nevertheless, long bone had proportionally thicker cortical part (80%) than trabecular bone (20%). During bone growth, the cortical part of long bone such as radius grow faster with rapid turnover of bone mineral density than the trabecular bone. In addition, Mazess et al. stated that calcium decreased the rate of cortical porosity for the treatment of osteoporosis in adult.<sup>(135)</sup> In this study, the effect of calcium intake was found on radius bone mass.

The influence of calcium intake on bone status has been debated in several studies.<sup>(8,15,72,73)</sup> The effect of calcium on bone mass was obviously seen in children with profound calcium deprivation, below 300 mg.d<sup>-1</sup>.<sup>(15)</sup> The small change of BMD was observed in children with 3-year supplements of 718 mg of calcium per day, whose dietary calcium intake of 900 mg.d<sup>-1</sup> was close to the recommendation.<sup>(8)</sup> Inversely, calcium intake was not associated with bone mass in children whose mean intake was greater than 1200 mg.<sup>(72)</sup> From those studies, calcium seemed to have a threshold effect on BMD. Therefore, calcium intake of the subjects were divided into 6 groups; 1 < 400; 2 = 401- 600; 3 = 601-800; 4 = 801-1000; 5 = 1001-1200; 6 > 1200 mg.d<sup>-1</sup>. The effect of calcium intake level on bone mass was emphasized particularly in study sites

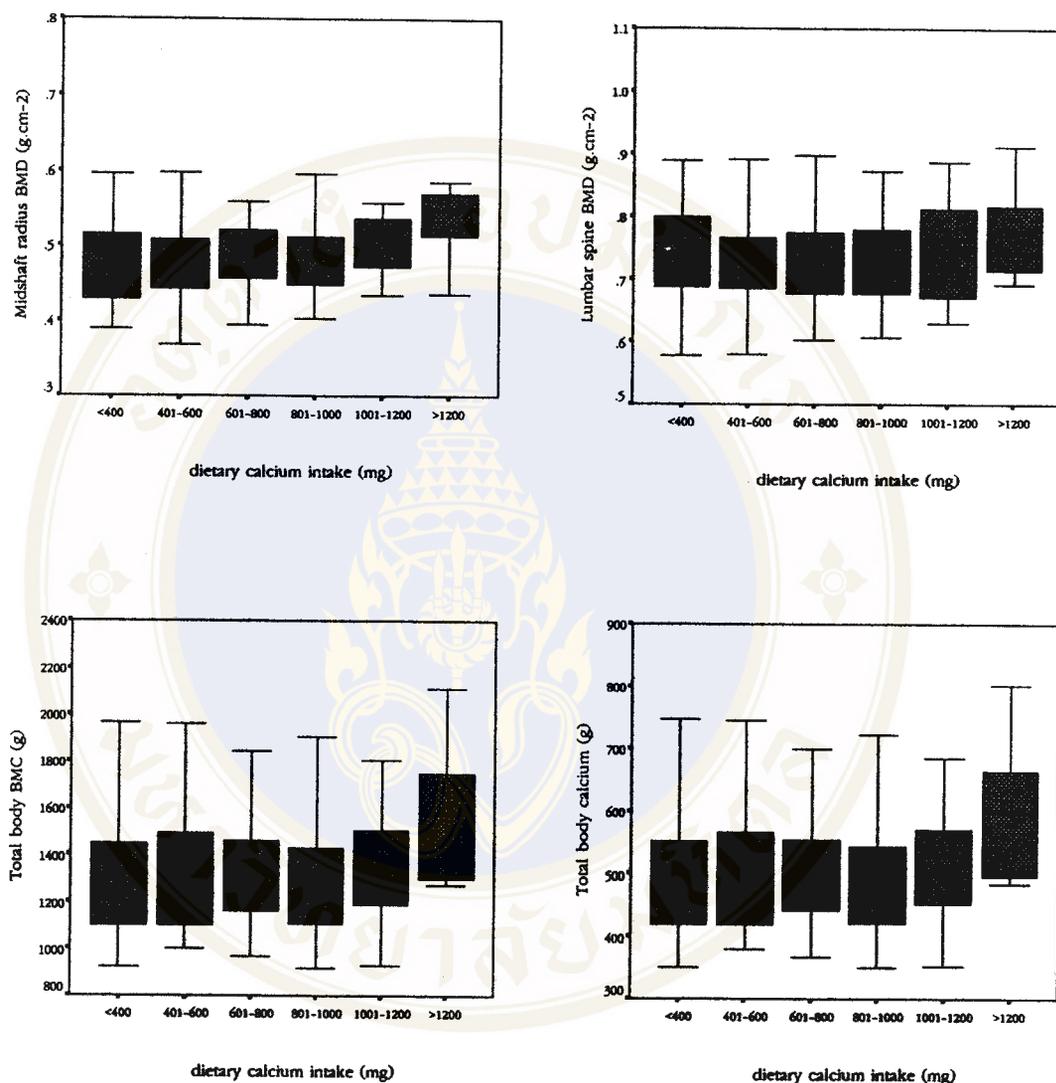
which were obviously changed such as midshaft radius BMD, lumbar spine BMD, total body BMC and total body calcium. It was shown that the level of calcium intake should be more than  $800 \text{ mg.d}^{-1}$  in both males and females (Figures 10 to 11).

Since pubertal stage had the major effect on bone mass, the relationship of calcium and bone status may be confounded by pubertal stage. By using Chi-square test, no relationship between puberty and the level of calcium intake was demonstrated. The result from this preliminary study demonstrated that Thai children should at least consumed calcium about  $800 \text{ mg.d}^{-1}$  to maintain bone mass gain.

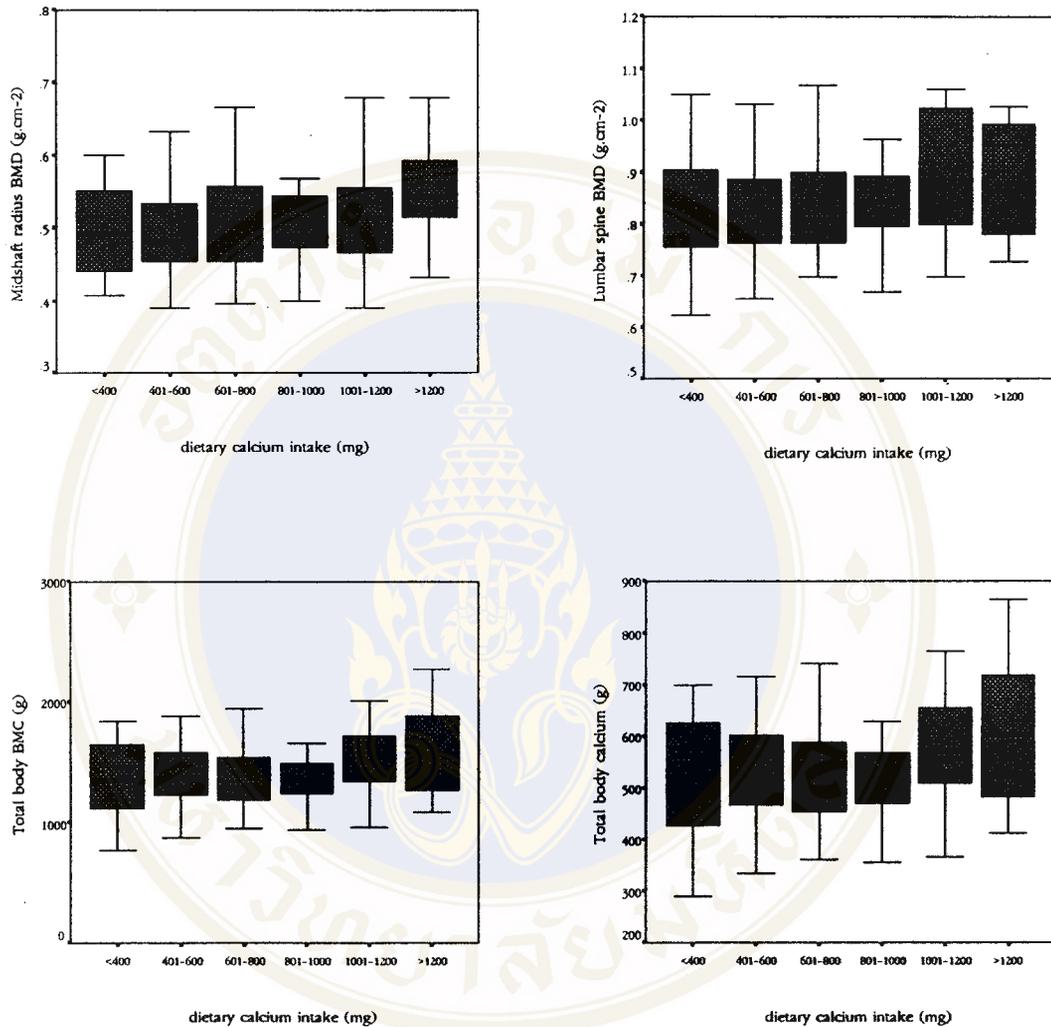
### **Weight bearing activity**

Although weight bearing activity was a minor determinant on bone mass. It was the environmental factor which was easily modified. There are evidences that physical activity during childhood had associated with adult bone density.<sup>(136)</sup> A cross-sectional exercise study demonstrated that trained post menopausal women generally had higher BMD than non-exercising women.<sup>(137)</sup> A woman exercising regularly throughout her life may have established greater bone density whereas another woman who was sedentary may have lower bone densities.<sup>(138,139)</sup> Exercising during the post menopausal years has been shown to slow the rate of bone loss and can improve bone mass following the completion of an exercise training program.<sup>(140)</sup>

Using the group mean value of time spent for weight bearing activity ( $6.5 \text{ hr.wk}^{-1}$ ) as a cut off point in this study, the effect of weight bearing activity was shown especially in males (Table 4.16-4.17). This result indicated that duration of weight bearing activity had benefit on bone mass gain in children whose time spent was greater



**Figure 10** Boxplot of bone mineral density (g.cm<sup>-2</sup>) at midshaft radius and lumbar spine, total body BMC (g) and total body calcium (g) against dietary calcium intake in male subjects. The top and bottom of each box represent the 75<sup>th</sup> and 25<sup>th</sup> percentile respectively; and the horizontal bar within each box represent the median value for the appropriate segment of the studied subjects.



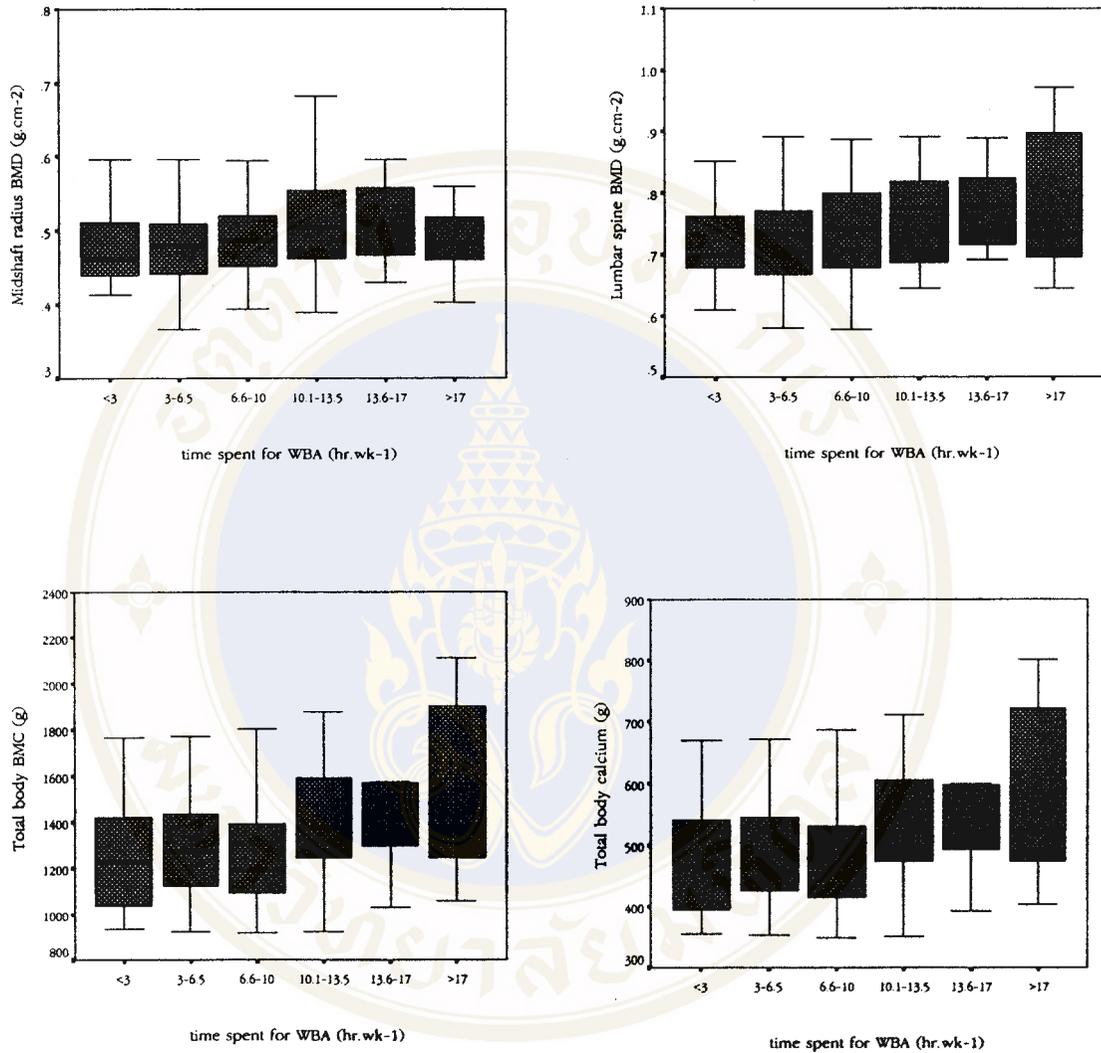
**Figure 11** Boxplot of bone mineral density (g.cm<sup>-2</sup>) at midshaft radius and lumbar spine, total body BMC (g) and total body calcium (g) against dietary calcium intake in female subjects. The top and bottom of each box represent the 75<sup>th</sup> and 25<sup>th</sup> percentile respectively; and the horizontal bar within each box represent the median value for the appropriate segment of the studied subjects.

than  $6.5 \text{ hr.wk}^{-1}$ . Among active group children who spent time for weight bearing activity  $>6.5 \text{ hr.wk}^{-1}$ , it was observed that males paid attention in playing football and basketball whereas females spent more time in table tennis and volleyball. Therefore, not only the duration of activity which had positive impact on bone mass gain but also type and intensity had an important role in regulation of bone mass.<sup>(88)</sup>

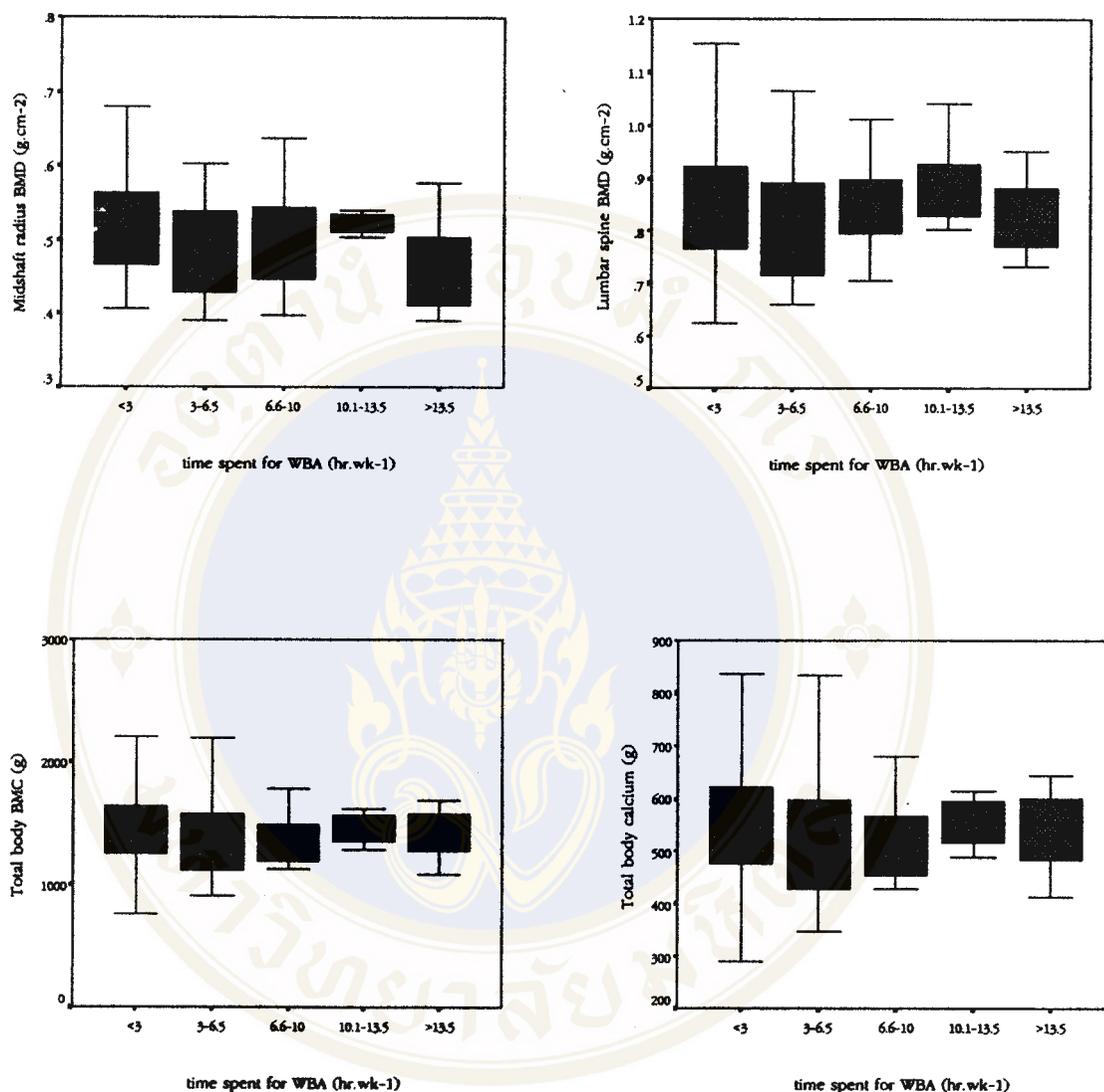
The same as calcium intake, this preliminary study was also conducted to identify the number of hours spent for weight bearing activity which might have impact on bone mass gain in children. Figure 12 and 13 showed that weight bearing activity at least  $6.5 \text{ hr.wk}^{-1}$  had beneficial effect on bone mass gain in both male and female subjects at lumbar spine, midshaft radius, total body BMC and total body calcium. The relationship between weight bearing activity and calcium and puberty was test by using Chi-square test. There was no the association between these variable.

Consideration with all factors which had beneficial effect on bone, some factors might be easily modified to promote bone mass gain such as weight, height, dietary calcium intake and physical activity but some was influenced by genetics which might have nothing to be done. However, some reports stated that pubertal stage could be developed through nutrition.<sup>(114)</sup> Bourguignon et al stated that the secular change in age at menarche have started earlier in well nourished population especially in developing countries.<sup>(142)</sup> The limitation of these factors might have an adverse effect on bone mass.

In conclusion, the major determinant on bone mass in Thai children was physiological factor (age, sex, puberty, weight, height) which described the variation on bone mass at all sites and total body. Although the environmental factor was the minor

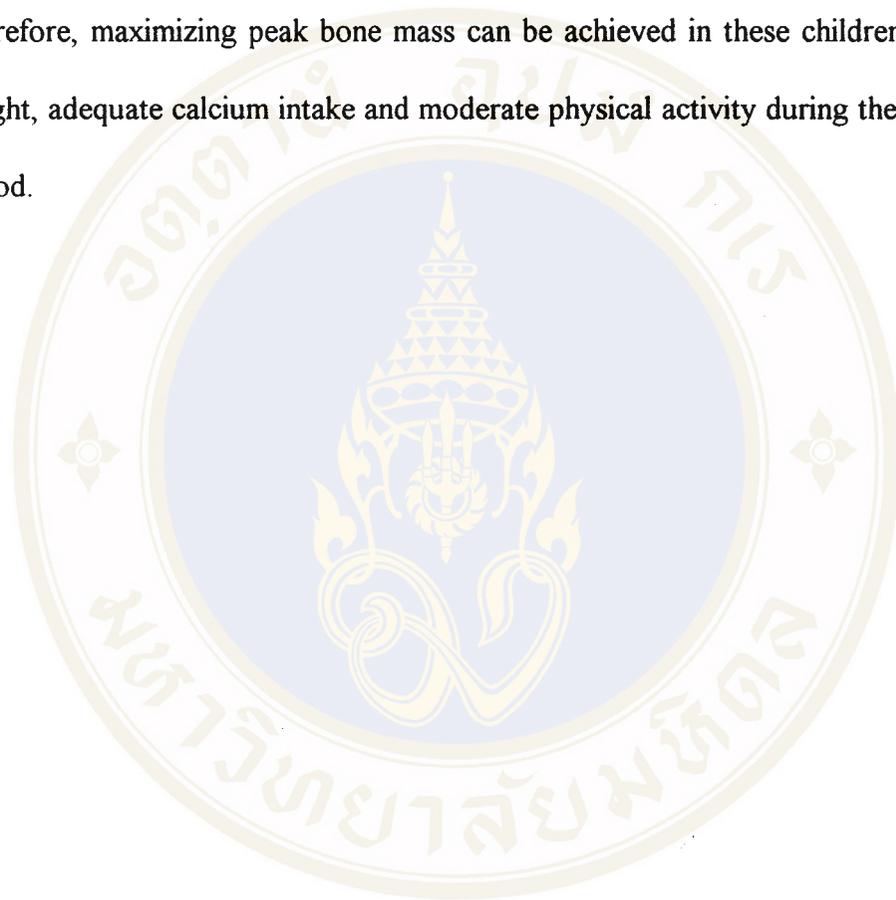


**Figure 12** Boxplot of bone mineral density (g.cm<sup>-2</sup>) at midshaft radius and lumbar spine, total body BMC (g) and total body calcium (g) against time spent for weight bearing activity in male subjects. The top and bottom of each box represent the 75<sup>th</sup> and 25<sup>th</sup> percentile respectively; and the horizontal bar within each box represent the median value for the appropriate segment of the studied subjects.



**Figure 13** Boxplot of bone mineral density ( $\text{g.cm}^{-2}$ ) at midshaft radius and lumbar spine, total body BMC (g) and total body calcium (g) against time spent for weight bearing activity in female subjects. The top and bottom of each box represent the 75<sup>th</sup> and 25<sup>th</sup> percentile respectively; and the horizontal bar within each box represent the median value for the appropriate segment of the studied subjects.

determinant, it can be modified to enhance the greater bone mass in the rapid growth period. The environmental factor (dietary calcium intake and weight bearing activity) had association with bone mass at different sites especially in subjects whose usual calcium intake and time spent for weight bearing activity was above their mean. Therefore, maximizing peak bone mass can be achieved in these children with proper weight, adequate calcium intake and moderate physical activity during the optimal time period.



## CHAPTER VI

### CONCLUSION

Maximizing peak bone mass during adolescent period has been suggested as one of the most important strategy in delaying rate of bone loss and eventually prevention of osteoporosis in the later years. However, there are several factors both controllable and uncontrollable which could determine the rate of bone gain during developmental years. Our results demonstrated that the Thai children and adolescent subjects were undergoing the developmental period both in physical growth and sexual maturation which has made it difficult to examine an independent effect of both physiological and environmental factors on their bone mass accretion. The differential effect of these factors on bone mass accretion at different bone sites was also demonstrated in this study. Noted that the physiological factors namely, age, sex, pubertal stage, body weight and height were the major determinants of bone growth especially on bone mass accretion in all skeletal sites : lumbar spine, femoral neck and midshaft radius as well as total body bone mass. The significant effect of dietary calcium intake on bone mass accretion was observed only at the midshaft radius and the effect of the number of hour per week of weight bearing activity was observed at lumbar spine site. Moreover, the effect of duration of weight bearing activity on bone mineralization was also evident by demonstrating a significant relationship with total body BMC and total body calcium.

Based on the results of our study, the optimum time to improve bone mass gain has been ascertained to be during the period of adolescence especially in females. The increment of bone mass in females was evident between the age of 11-12 years whereas the period of bone mass accretion in males was postponed until the age of 12 years. In addition, as children approached pubertal period, the rate of bone gain also increased as compared to pre-pubertal period. Among our study population, females had higher total body BMD and the BMD at lumbar spine than males. A similar result was also observed for body weight and height. As weight for height increased, the positive change of bone mass value for total body and at all bone sites was clearly seen.

Although a national campaign to increase milk consumption among Thai population especially among school children and women aiming at improvement of general nutritional status and prevention of osteoporosis was successfully increasing the awareness among the Thai population, ninety percent of the children and adolescent subjects in this study received dietary calcium below the RDA recommendation. Milk and milk product was found to be the major source of dietary calcium which contributed to approximately 57% of their total dietary calcium intake. Other sources of calcium were also from fish and aquatic animals, vegetables and cereals but the contribution from these food groups was very low (12%, 5% and 5%, respectively). Children who consumed higher amount of dietary calcium had greater bone mass than those who consumed less particularly at the midshaft radius site. To have benefit on bone mass gain, usual calcium intake should be at least 669 mg.d<sup>-1</sup>.

Weight bearing activity was another significant environmental factor which could positively determine the variation of bone mass in our study subjects especially at the lumbar spine which is a weight bearing site. It also affected the total body BMC and total body calcium. To have a significant effect on bone mass accretion or rate of bone gain, one has to spend at least 6.5 hours per week for weight bearing activity.

In conclusion, results obtained from this study clearly demonstrated that the peak bone mass increment started at the earlier age (9-12 years) particularly in females who have a greater risk of developing osteoporosis during their elderly years. The age at which peak bone mass is achieved is not certain. It is therefore an important issue to maximize the peak bone mass in children as early as the period of pre-puberty by taking into account for the modification of the environmental factor under the influence of physiological factor. Although the influence of calcium intake on bone mass at different bone sites remains uncertain, a profound effect of usual calcium intake on bone mass accretion was observed at the midshaft radius site which can be interpreted that the peak bone mass at midshaft radius site may started at earlier age. Increasing dietary calcium intake during the early adolescent years can ameliorate the bone mass accretion at this specific bone site. To prevent osteoporosis through an improvement of bone status early in life, we should promote good dietary habit and life style practice through maintenance of appropriate weight, higher consumption of calcium rich food to meet RDA level throughout the period of childhood and adolescence and regular physical activity especially weight bearing activity for at least 1 hour per day to ensure an additional beneficial effect on bone gain.

The result from this study will lay down the basic information and ideas for the further research to prevent the development of early osteoporosis and incidental fracture in later life.

### **Recommendation for future research**

Our result demonstrated a differential positive relationship between calcium intake and bone mass accretion at a variety of bone sites. Therefore, it is advised to increase the calcium intake proportionally to the time of bone growth and development. Further study to determine the amount and form of calcium intake to maximize peak bone mass among children and adolescents as well as an appropriate time during phases of growth in increasing calcium intake to benefit peak bone mass acquisition is essential. Another challenging area of research is to determine the beneficial effect of duration and type as well as an intensity of weight bearing activity on bone mass accretion during adolescent period. Furthermore, a research to establish an effective strategy in improving bone status among Thai population is required to prevent the development of osteoporosis in later life.

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## APPENDIX A

### **The pubertal stage of males**

The staging of male pubertal sexual maturation is based on genital size and pubic hair development.

#### ***The stage of genital size***

Stage 1: Preadolescence. testes, scrotum and penis are of about the same size and proportion as in early children.

Stage 2: The scrotum and testes have enlarged and there is a change in the texture of the scrotal skin. There is also some reddening of the scrotal skin. The length of the longitudinal axis of the testes ranges from 2.5-3.2 cm. Age onset of puberty is characterized by appearance of testicular growth by which a testis longer than 2.5 cm for male and the physical breast budding (the larche) in the stage 2 of breast development for female.

Stage 3: Growth of the penis has occurred, at first mainly in length but with some increase in breadth. There has been further growth of testes and scrotum and the testes have increased in the length to 3.3-4.0 cm.

Stage 4 : Penis further enlarged in length and breadth with development of glands. Testes and scrotum further enlarged. There is also further darkening of the scrotal skin. The long axis of the testes is 4.0-4.5 cm.

Stage 5 : Genitalia adult in size and shape. No further enlargement takes place after stages is reached.

## **The pubertal stage of females**

The staging of female pubertal sexual maturation is based on breast size

### ***The stage of breast size***

Stage 1 : The preberal stage no palpable breast tissue, with areola generally less than 2 cm. In diameter and may be relatively flat, inverted, or raised, although the papillae may be slightly raised from the chest contour.

Stage 2 : There is a visible and palpable mound of breast and papillae with increased areola diameter. The skin of the areola becomes thinner. The nipple may be developed to various degrees.

Stage 3 : Further enlargement of breast and areola, with no separation of their contour.

Stage 4 : Projection of areola and papilla to form a secondary mound above the level of the breast.

Stage 5 : Mature stage, projection of papilla only, due to recession of the areola to the general contour of the breast.

In this study, we categorized the pubertal stage into three subgroups as the following :

Pre-puberty was defined as pubertal stage 1.

Early-puberty was defined as pubertal stages 2 and 3.

Late-puberty was defined as pubertal stage 4 and 5.

## APPENDIX B

โครงการศึกษาภาวะโภชนาการของแคลเซียมและความหนาแน่นของกระดูกในเด็กไทย อายุ 9-18 ปี  
คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี และ สถาบันวิจัยโภชนาการ  
มหาวิทยาลัยมหิดล

### แบบสอบถามความถี่ในการบริโภคอาหาร

ชื่อ ..... นามสกุล ..... เพศ .....  
โรงเรียน ..... ชั้น ..... วันที่ตอบแบบสอบถาม .....

ข้อมูลความถี่ในการรับประทานอาหารในช่วง 6 เดือนที่ผ่านมา

ชื่ออาหาร	ปริมาณ หรือ น้ำหนักที่ รับ ประทาน ต่อครั้ง	จำนวนครั้งโดยเฉลี่ย								
		ไม่เคย หรือ < 1 ครั้ง ต่อ เดือน	1 ครั้ง ต่อ เดือน	2-3 ครั้ง ต่อ เดือน	1-2 ครั้ง ต่อ สัปดาห์	3-4 ครั้ง ต่อ สัปดาห์	5-6 ครั้ง ต่อ สัปดาห์	1 ครั้ง ต่อ วัน	2-3 ครั้ง ต่อ วัน	≥ 4 ครั้ง ต่อ วัน
<b>1. กลุ่มนมและผลิตภัณฑ์</b>										
<b>1.1 นม (ทุกชนิด)</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- นมสด/นมจืด/ นมผงผสม	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- นมปรุงแต่งรส	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- นมสดพร้อม ไขมัน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>1.2 ผลิตภัณฑ์นม (ทุกชนิด)</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- โยเกิร์ต/ไอวอลล์ (ชนิดกล่อง)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- โยเกิร์ต (ครีม)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- นมเปรี้ยวชนิด ดื่ม	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เนยแข็ง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- นมอัสเม็ค	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆระบุ.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

ชื่ออาหาร	ปริมาณ หรือ น้ำหนักที่ รับประทาน ต่อครั้ง	จำนวนครั้งโดยเฉลี่ย								
		ไม่เค หรือ < 1 ครั้ง ต่อ เดือน	1 ครั้ง ต่อ เดือน	2-3 ครั้ง ต่อ เดือน	1-2 ครั้ง ต่อ สัปดาห์	3-4 ครั้ง ต่อ สัปดาห์	5-6 ครั้ง ต่อ สัปดาห์	1 ครั้ง ต่อ วัน	2-3 ครั้ง ต่อ วัน	≥ 4 ครั้ง ต่อ วัน
<b>2. กลุ่มเนื้อสัตว์ไข่ และผลิตภัณฑ์</b>										
<b>2.1 เนื้อสัตว์ทุก ชนิด (ยกเว้นปลา และอาหารทะเล)</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เนื้อวัว	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- หมูเนื้อแดง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- หมูสามชั้น	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- หมูสับ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ชีโครงหมู	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เนื้อไก่ไม่มีหนัง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เนื้อไก่มีหนัง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เนื้อเป็ดไม่มีหนัง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เนื้อเป็ดมีหนัง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>2.2 เครื่องในสัตว์ (ทุกชนิด)</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ตับ(วัว หมู เป็ด ไก่)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เลือด(วัว หมู เป็ด ไก่)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เครื่องในอื่นๆ เช่น เซ่งจี้ (ไต) หัวใจ ม้าม	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>2.3 ผลิตภัณฑ์จาก เนื้อสัตว์</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ลูกชิ้น (วัว หมู ไก่)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ลูกชิ้นปลา	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ไส้กรอกหมู ไก่ (แห้ง)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ไส้กรอกหมู ไก่ (แฉ่น)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- กุนเชียง(หมู ไก่)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

ชื่ออาหาร	ปริมาณ หรือ น้ำหนักที่ รับประทาน ต่อครั้ง	จำนวนครั้งโดยเฉลี่ย								
		ไม่เคย หรือ < 1 ครั้ง ต่อ เดือน	1 ครั้ง ต่อ เดือน	2-3 ครั้ง ต่อ เดือน	1-2 ครั้ง ต่อ สัปดาห์	3-4 ครั้ง ต่อ สัปดาห์	5-6 ครั้ง ต่อ สัปดาห์	1 ครั้ง ต่อ วัน	2-3 ครั้ง ต่อ วัน	≥ 4 ครั้ง ต่อ วัน
- แสม	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เบคอน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- หมูหยอง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- หมูแผ่น	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- หมูขย	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- แหนม	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>2.4 ไข่ (ทุกชนิด)</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ไข่เป็ด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ไข่ไก่	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ไข่นกกระทา	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>3. ปลา</b>										
<b>3.1 ปลา (ทุกชนิด)</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เนื้อปลา เช่น	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
ปลาช่อน ปลา	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
กะพง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ปลารับประทาน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
ได้ทั้งเนื้อและ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
กระดูก เช่น ปลา	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
เล็กปลาน้อย	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
ปลาไส้ตัน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ปลากระป๋องใน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
ซอสมะเขือเทศ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ปลาเกล็ดขาว	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
กระป๋อง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>4. อาหารทะเลสด</b>										
<b>4.1 อาหารทะเล (ทั้งหมด)</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- กุ้งสด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

ชื่ออาหาร	ปริมาณ หรือ น้ำหนักที่ รับ ประทาน ต่อครั้ง	จำนวนครั้งโดยเฉลี่ย								
		ไม่เคย หรือ < 1 ครั้ง ต่อ เดือน	1 ครั้ง ต่อ เดือน	2-3 ครั้ง ต่อ เดือน	1-2 ครั้ง ต่อ สัปดาห์	3-4 ครั้ง ต่อ สัปดาห์	5-6 ครั้ง ต่อ สัปดาห์	1 ครั้ง ต่อ วัน	2-3 ครั้ง ต่อ วัน	≥ 4 ครั้ง ต่อ วัน
- เนื้อปู	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ปลาหมึกสด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- หอยต่างๆ เช่น หอยแครง หอย แมลงงู หอยลาย	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>5. อาหารทะเล</b>										
<b>แห้ง</b>										
<b>5.1 อาหารทะเล</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>แห้ง (ทั้งหมด)</b>										
- กุ้งแห้ง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ปลาหมึกแห้ง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>6. กลุ่ม ข้าว แป้ง</b>										
<b>และผลิตภัณฑ์</b>										
<b>6.1 ข้าว</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ข้าวเจ้า	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ข้าวเหนียว	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>6.2 แป้ง</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมหิน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- กว๊าดข้าวแต๋น	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>ใหญ่</b>										
- กว๊าดข้าวแต๋นเล็ก	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เส้นหมี่ขาว	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เส้นบะหมี่	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- บะหมี่เส้นเรีจรูป	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- มักกะโรนี	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ตปนีกัดดี	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- รุ้นเส้น	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

ชื่ออาหาร	ปริมาณ หรือ น้ำหนักที่ รับประทาน ต่อครั้ง	จำนวนครั้งโดยเฉลี่ย								
		ไม่เคย หรือ < 1 ครั้ง ต่อ เดือน	1 ครั้ง ต่อ เดือน	2-3 ครั้ง ต่อ เดือน	1-2 ครั้ง ต่อ สัปดาห์	3-4 ครั้ง ต่อ สัปดาห์	5-6 ครั้ง ต่อ สัปดาห์	1 ครั้ง ต่อ วัน	2-3 ครั้ง ต่อ วัน	≥ 4 ครั้ง ต่อ วัน
<b>6.3 ผลิตภัณฑ์จาก ข้าว แป้ง และ ธัญพืชอื่นๆ</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อาหารสำเร็จรูป เช่น คอร์นเฟลก		.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมปังต่างๆ (แผ่น)		.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมปังโฮลวีท		.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมปังกรอบ แบบหวาน เช่น บิสกิต		.....	.....	.....	.....	.....	.....	.....	.....	.....
- แครกเกอร์		.....	.....	.....	.....	.....	.....	.....	.....	.....
- ข้าวโพคขาว/ เหลือง (ฝัก)		.....	.....	.....	.....	.....	.....	.....	.....	.....
- เผือก, มันเทศ		.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ..... .....		.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>7. ถั่ว พืชเมล็ด และผลิตภัณฑ์</b>		.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>7.1 เต้าหู้ (ทุกชนิด)</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เต้าหู้แข็ง		.....	.....	.....	.....	.....	.....	.....	.....	.....
- เต้าหู้ขาวอ่อน		.....	.....	.....	.....	.....	.....	.....	.....	.....
- เต้าหู้หลอด		.....	.....	.....	.....	.....	.....	.....	.....	.....
- น้ำเต้าหู้/นมถั่ว เหลือง		.....	.....	.....	.....	.....	.....	.....	.....	.....
- เต้าฮวย		.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ..... .....		.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>7.2 ถั่วอื่นๆ</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ถั่วเขียว		.....	.....	.....	.....	.....	.....	.....	.....	.....
- ถั่วแดง		.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ..... .....		.....	.....	.....	.....	.....	.....	.....	.....	.....

ชื่ออาหาร	ปริมาณ หรือ น้ำหนักที่ รับประทาน ต่อครั้ง	จำนวนครั้งโดยเฉลี่ย								
		ไม่เคย หรือ < 1 ครั้ง ต่อ เดือน	1 ครั้ง ต่อ เดือน	2-3 ครั้ง ต่อ เดือน	1-2 ครั้ง ต่อ สัปดาห์	3-4 ครั้ง ต่อ สัปดาห์	5-6 ครั้ง ต่อ สัปดาห์	1 ครั้ง ต่อ วัน	2-3 ครั้ง ต่อ วัน	≥ 4 ครั้ง ต่อ วัน
<b>7.3 พืชเมล็ด และ ผลิตภัณฑ์</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- งาคั่ว/ขาว	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เมล็ดพืชทองแห้ง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เมล็ดอัลมอนต์	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เมล็ดมะม่วง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
หิมพานต์	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ถั่วลิสงอบกรอบ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
เช่น โกโก้ ซอบบี้	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ถั่วลิสงเคลือบกรอบ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
เช่น กรีนนัท	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>8. ผัก</b>										
<b>8.1 ผักกินดิบ</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- แดงกวา	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ถั่วฝักยาว	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- กะหล่ำปลี	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ผักกาดหอม	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- มะเขือเทศ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- มะเขือเปราะ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- กระถิน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ผักบุ้งไทย	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ถั่วงอก	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- แครอท	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- มะละกอ(ดิบ)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ใบโหระพา	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>8.2 ผักกินสุก</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- คะน้า	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- กะหล่ำปลี	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ผักบุ้งไทย/จีน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ถั่วฝักยาว	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ถั่วงอก	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

ชื่ออาหาร	ปริมาณ หรือ น้ำหนักที่ รับประทาน ต่อครั้ง	จำนวนครั้งโดยเฉลี่ย								
		ไม่เคย หรือ < 1 ครั้ง ต่อ เดือน	1 ครั้ง ต่อ เดือน	2-3 ครั้ง ต่อ เดือน	1-2 ครั้ง ต่อ สัปดาห์	3-4 ครั้ง ต่อ สัปดาห์	5-6 ครั้ง ต่อ สัปดาห์	1 ครั้ง ต่อ วัน	2-3 ครั้ง ต่อ วัน	≥ 4 ครั้ง ต่อ วัน
- เห็ด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ผักกาดเขียว/ ขาว	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ผักโขม	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ชะอม	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ผักกะเฉด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ใบกระเพรา	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ใบยอ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ใบชะพลู	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ถั่วลิสง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ลำไย	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- มะเขือยาว	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- มะเขือเปราะ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- มะเขือเทศ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ใบขี้เหล็ก	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- แตงกวา	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ฟัก	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ฟักทอง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- หน่อไม้	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- บวบเหลี่ยม	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ผักกวางตุ้ง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ดอกกระหล่ำ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- กุยช่าย	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ข้าวโพดอ่อน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- แครอท	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- บรอกโคลี	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- มะละกอ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>9. ผลไม้</b>										
<b>9.1 ผลไม้สด</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
(ทุกชนิด)										
- ส้มเขียวหวาน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ส้มโอ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- กล้วยไข่	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

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- กลัวยน้ำว้า	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- กลัวยหอม	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- สับปะรด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ฝรั่ง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- แดงโม	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- มะละกอสุก	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- แอปเปิ้ล	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ชมพู	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- มะม่วงดิบ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- มะม่วงสุก	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เงาะ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ทุเรียน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- มะขามหวาน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ลำไย	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- มังคุด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- องุ่น	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนุน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>9.2 ผลไม้</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>กระป๋อง</b>		.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>(ทุกชนิด)</b>		.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>ระบุชนิด</b>		.....	.....	.....	.....	.....	.....	.....	.....	.....
1. ....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
2. ....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
3. ....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>10. อาหารว่าง</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมหีบ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- จาละเป่าไส้เค็ม	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- จาละเป่าไส้	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
หวาน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- สาเกไส้หมู	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ข้าวเกรียบปาก	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
หม้อ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมหุ่ย	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

ชื่ออาหาร	ปริมาณ หรือ น้ำหนักที่ รับ ประทาน ต่อครั้ง	จำนวนครั้งโดยเฉลี่ย								
		ไม่เคย หรือ < 1 ครั้ง ต่อ เดือน	1 ครั้ง ต่อ เดือน	2-3 ครั้ง ต่อ เดือน	1-2 ครั้ง ต่อ สัปดาห์	3-4 ครั้ง ต่อ สัปดาห์	5-6 ครั้ง ต่อ สัปดาห์	1 ครั้ง ต่อ วัน	2-3 ครั้ง ต่อ วัน	≥ 4 ครั้ง ต่อ วัน
- ก๋วยเตี๋ยวหลอด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เต้าหู้ทอด/ เผือกทอด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ข้าวต้มมัด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ไข่กรอกอีสาน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ข้าวตังหมูหยอง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ทอดมัน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ปอเปี๊ยะทอด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ปอเปี๊ยะสด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆระบุ..... .....	..... .....	..... .....	..... .....	..... .....	..... .....	..... .....	..... .....	..... .....	..... .....	..... .....
<b>11. ขนมหวาน</b>										
<b>11.1 ขนมหวาน ไทย</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมทองม้วน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมฉิ่ง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมปุยฝ้าย	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมสาลี่	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมเปียกปูน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมถั่วแปบ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมลูกชุบ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมกล้วย	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมเทียน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมครก	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมอั่ว	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ผลไม้กวนต่างๆ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ぜんท้อว เหือก อั่ว สับปะรด ทุเรียน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ..... .....	..... .....	..... .....	..... .....	..... .....	..... .....	..... .....	..... .....	..... .....	..... .....	..... .....

ชื่ออาหาร	ปริมาณ หรือ น้ำหนักที่ รับประทาน ต่อครั้ง	จำนวนครั้งโดยเฉลี่ย								
		ไม่เคย หรือ < 1 ครั้ง ต่อ เดือน	1 ครั้ง ต่อ เดือน	2-3 ครั้ง ต่อ เดือน	1-2 ครั้ง ต่อ สัปดาห์	3-4 ครั้ง ต่อ สัปดาห์	5-6 ครั้ง ต่อ สัปดาห์	1 ครั้ง ต่อ วัน	2-3 ครั้ง ต่อ วัน	≥ 4 ครั้ง ต่อ วัน
<b>11.3 ขนมเค้ก</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ถั่วคาน้ำกะทิ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ข้าวเหนียวถั่วดำ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ข้าวเหนียวเปียก / สาขุเปียก	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เต้าส่วน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ฟักทองแกงบวด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- มันแกงบวด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เสื่อกแกงบวด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- กุ้งขบวดซี	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- บัวลอย	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>11.4 ผลไม้พิช หัวเชื่อม</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- กุ้งเชื่อม	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- มันสำปะหลัง เชื่อม	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- มันเทศเชื่อม	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>11.5 ข้าวเหนียว หน้าต่างๆ</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- หน้ากุ้ง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- หน้าสังขยา	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- หน้ากระฉิก	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- หน้าปลาแห้ง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ข้าวเหนียวแก้ว	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>11.6 ของหวาน ปรุงงาไข่</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ทองหยิบ/ ทองหยอด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

ชื่ออาหาร	ปริมาณ หรือ น้ำหนักที่ รับประทาน ต่อครั้ง	จำนวนครั้งโดยเฉลี่ย								
		ไม่เคย หรือ < 1 ครั้ง ต่อ เดือน	1 ครั้ง ต่อ เดือน	2-3 ครั้ง ต่อ เดือน	1-2 ครั้ง ต่อ สัปดาห์	3-4 ครั้ง ต่อ สัปดาห์	5-6 ครั้ง ต่อ สัปดาห์	1 ครั้ง ต่อ วัน	2-3 ครั้ง ต่อ วัน	≥ 4 ครั้ง ต่อ วัน
- ผอ่ยทอง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เม็ดขนุน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>11.7 ผอ่ยไม้พื้</b>	<b>X</b>	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>หัว เป็งทอด</b>		.....	.....	.....	.....	.....	.....	.....	.....	.....
- กล้วยแขก	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- มันทอด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมน ไข่กระทา	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ปาท่องโก๋	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>11.8 ผอื่คัณฑ์</b>	<b>X</b>	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>ขนมอบ</b>		.....	.....	.....	.....	.....	.....	.....	.....	.....
- เค้กหน้าครีม	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เค้กหน้าแยม/ เค้กกล้วยหอม/ เค้กเนย/แชนโรล	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- คุกกี้	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมพาย (ไส้เค็ม)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- พายผลไม้ เช่น พายลูกตาล พายสับปะรด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- แอแคล	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- โคนัท	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- คุโรคัสตาร์ดเค้ก	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เลเซอร์เค้ก	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมปังไส้หมูสับ /หมูหยอง/หมู แดง/ไก่	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมปังไส้กรอก /แฮม	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมปังไส้ผลไม้ (ลูกเกด/เชอรี่)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมปังเนยสด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

ชื่ออาหาร	ปริมาณ หรือ น้ำหนักที่ รับประทาน ต่อครั้ง	จำนวนครั้งโดยเฉลี่ย								
		ไม่เคย หรือ < 1 ครั้ง ต่อ เดือน	1 ครั้ง ต่อ เดือน	2-3 ครั้ง ต่อ เดือน	1-2 ครั้ง ต่อ สัปดาห์	3-4 ครั้ง ต่อ สัปดาห์	5-6 ครั้ง ต่อ สัปดาห์	1 ครั้ง ต่อ วัน	2-3 ครั้ง ต่อ วัน	> 4 ครั้ง ต่อ วัน
<b>11.9 ไอศกรีม</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>ชอคโกแลต</b>		.....	.....	.....	.....	.....	.....	.....	.....	.....
- ไอศกรีมกะทิ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ไอศกรีมหวาน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
เช่น		.....	.....	.....	.....	.....	.....	.....	.....	.....
- ไอศกรีมชอร์เบต	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ไอศกรีมรสอื่นๆ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
เช่นวานิลลา		.....	.....	.....	.....	.....	.....	.....	.....	.....
สตอร์เบอร์รี่		.....	.....	.....	.....	.....	.....	.....	.....	.....
ชอคโกแลต		.....	.....	.....	.....	.....	.....	.....	.....	.....
- ชอคโกแลต	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
แท่ง/ เม็ด		.....	.....	.....	.....	.....	.....	.....	.....	.....
- ชอคโกแลต	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
แท่งเม็ดสอดไส้		.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>12. ขนมขบเคี้ยว</b>		.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>12.1 ขนมขบ</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>เคี้ยว</b>		.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>(ทั้งหมด)</b>		.....	.....	.....	.....	.....	.....	.....	.....	.....
- มันฝรั่งแป้งมัน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
ฝรั่งอบกรอบ		.....	.....	.....	.....	.....	.....	.....	.....	.....
เช่น โปเตโตชิป		.....	.....	.....	.....	.....	.....	.....	.....	.....
มันมัน เลย์		.....	.....	.....	.....	.....	.....	.....	.....	.....
- มันเทศอบเนย	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
คาราเมล เช่น		.....	.....	.....	.....	.....	.....	.....	.....	.....
ปาร์ตี้		.....	.....	.....	.....	.....	.....	.....	.....	.....
- ข้าวเกรียบกุ้ง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ข้าวเกรียบรส	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
ต่างๆ		.....	.....	.....	.....	.....	.....	.....	.....	.....

ชื่ออาหาร	ปริมาณ หรือ น้ำหนักที่ รับประทาน ต่อครั้ง	จำนวนครั้งโดยเฉลี่ย								
		ไม่เคย หรือ < 1 ครั้ง ต่อ เดือน	1 ครั้ง ต่อ เดือน	2-3 ครั้ง ต่อ เดือน	1-2 ครั้ง ต่อ สัปดาห์	3-4 ครั้ง ต่อ สัปดาห์	5-6 ครั้ง ต่อ สัปดาห์	1 ครั้ง ต่อ วัน	2-3 ครั้ง ต่อ วัน	≥ 4 ครั้ง ต่อ วัน
- แป้งข้าวสาลี	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
ข้าว/ข้าวเหนียว	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
ข้าวโพดอบ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
กรอบเช่น โครเมี	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
ปูซอปูเพื่อ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
ริงโกตตาร์	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมปังแห้ง เช่น	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
กูลิโกะฟรายด์	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
พริทซ์	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมปังแห้ง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
เคลือบรสต่างๆ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
เช่นกูลิโกะ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
เคลือบ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
สตรอเบอร์รี่	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมปังเวเฟอร์	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมปังกรอบ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
สอคใต้ เช่น	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
โคลอน ทินนี่	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
นัทเชล	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมหวานรส	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
ต่างๆ เช่น รสนม	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
รสชอคโกแลต	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
อัลฟี แคลิฟอร์	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
เนียบาร์	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ปลาน้ำเค็ม เช่น	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
ทาโร เบนโตะ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เชลลี	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ขนมถั่วลิสงเตา	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
เช่น ตมนกแจ๊ก	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>13. ฟาสต์ฟู้ด</b>										
<b>13.1 ฟาสต์ฟู้ด</b>	X									
(ทั้งหมด)										
- แฮมเบอร์เกอร์	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ชีสเบอร์เกอร์	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- พิซซ่า	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

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- ไม้ทอด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- แจนคัวย	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เฟรนช์ฟราย	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ซอทอด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- มันบด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- หอมทอด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- โคลกถลอว์	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>14. เครื่องดื่ม</b>										
<b>14.1 น้ำผลไม้สด</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- น้ำส้มคั้น	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- น้ำมะนาว	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- น้ำฝรั่ง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>14.2 น้ำผลไม้ กระป๋อง</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- น้ำส้ม	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- น้ำสับปะรด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- น้ำอุนุ่น	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>14.3 น้ำผลไม้ ผสม</b>										
เช่น น้ำแก้ว										
น้ำมะพร้าว										
น้ำลูกกอด										
<b>14.4 น้ำอัดลม</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
(ทั้งหมด)										
- น้ำอัดลมแบบ	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
โคล่า เช่น เป๊ปซี่	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

ชื่ออาหาร	ปริมาณ หรือ น้ำหนักที่ รับประทาน ต่อครั้ง	จำนวนครั้งโดยเฉลี่ย								
		ไม่เคย หรือ < 1 ครั้ง ต่อ เดือน	1 ครั้ง ต่อ เดือน	2-3 ครั้ง ต่อ เดือน	1-2 ครั้ง ต่อ สัปดาห์	3-4 ครั้ง ต่อ สัปดาห์	5-6 ครั้ง ต่อ สัปดาห์	1 ครั้ง ต่อ วัน	2-3 ครั้ง ต่อ วัน	≥ 4 ครั้ง ต่อ วัน
<b>14.6 เครื่องดื่ม ร้อนเย็น (ทั้งหมด)</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ผงไมโล	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ผงโอวัลติน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เมื่อดื่มเครื่องดื่ม ดังกล่าวบอก ปริมาณของสิ่งต่อ ไปนี้ (ถ้าดื่ม)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- นมสด	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- นมข้นหวาน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- คริมเทียม	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>14.7 เครื่องดื่ม ทั่วไป</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ชาอู่หลง	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- บั๊ทานแกน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆระบุ.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
<b>15. กลุ่มอื่นๆ</b>	X	.....	.....	.....	.....	.....	.....	.....	.....	.....
- แขน	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- เนย	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- ตั้งขยา	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- สลัดน้ำข้น	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- อื่นๆ ระบุ .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

### APPENDIX C

โครงการศึกษาภาวะโภชนาการของแมลงเชื่อมและความหนาแน่นของกระดูกในเด็กไทย อายุ 9-18 ปี  
 คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี และ สถาบันวิจัยโภชนาการ

มหาวิทยาลัยมหิดล

Checks by

\_\_\_\_\_

\_\_\_\_\_/\_\_\_\_

Code.....

Initial _____	Birth date ____/____/____	Sex M      F
------------------	------------------------------	-----------------

#### แบบสอบถามกิจกรรมและการออกกำลังกาย

ชื่อ..... นามสกุล..... เพศ.....  
 โรงเรียน..... ชั้น..... วันที่ตอบแบบสอบถาม.....

1. ปกตินักเรียนมาโรงเรียนโดย

- |   |                             |
|---|-----------------------------|
| <input type="checkbox"/> รถ (ทุกประเภท)   | ใช้เวลาเดินทาง..... ชั่วโมง |
| <input type="checkbox"/> เดิน             | ใช้เวลาเดิน..... ชั่วโมง    |
| <input type="checkbox"/> อื่น ๆ ระบุ..... | ใช้เวลาเดินทาง..... ชั่วโมง |
| เดินทางกลับบ้านโดย                        |                             |
| <input type="checkbox"/> รถ (ทุกประเภท)   | ใช้เวลาเดินทาง..... ชั่วโมง |
| <input type="checkbox"/> เดิน             | ใช้เวลาเดิน..... ชั่วโมง    |
| <input type="checkbox"/> อื่น ๆ ระบุ..... | ใช้เวลาเดินทาง..... ชั่วโมง |

2. นักเรียนมีวิชาเรียนที่เกี่ยวกับพลศึกษาหรือการออกกำลังกายเป็นประจำหรือไม่

- |  |   |
|--|---|
| <input type="checkbox"/> ไม่มี                 | <input type="checkbox"/> มี               |
| ถ้ามี <input type="checkbox"/> 1 ครั้ง/สัปดาห์ | <input type="checkbox"/> 2 ครั้ง/สัปดาห์  |
| <input type="checkbox"/> 3 ครั้ง/สัปดาห์       | <input type="checkbox"/> อื่น ๆ ระบุ..... |

ถ้าไม่มี ให้ข้ามไปตอบในข้อ 7 ได้เลย

3. กิจกรรมที่นักเรียนทำในวิชาพลศึกษา เช่น กายบริหาร วายน้ำ แบดมินตัน ฯลฯ

(ให้บอกกิจกรรมทุกอย่างที่ทำในวิชาพลศึกษา)

.....

.....

4. เวลาที่ใช้ในการเรียนวิชาพลศึกษาประมาณ

- |   |  |  |
|---|--|--|
| <input type="checkbox"/> 30 นาที/ครั้ง    | <input type="checkbox"/> 45 นาที/ครั้ง | <input type="checkbox"/> 60 นาที/ครั้ง |
| <input type="checkbox"/> อื่น ๆ ระบุ..... |  |  |

5. ช่วงเวลาที่เรียนวิชาพลศึกษาคือ

ช่วงเช้า..... นาฬิกา

ช่วงบ่าย..... นาฬิกา

6. สถานที่ที่เรียนพลศึกษา

กลางแจ้ง

ในร่ม

ทั้งกลางแจ้งและในร่ม

7. กิจกรรมใดบ้างที่นักเรียนได้ทำอยู่นอกเหนือจากเวลาเรียนพลศึกษา (ตอบได้มากกว่า 1 ข้อ)

และใช้เวลาเท่าใด

กิจกรรม	เวลาที่ใช้ (ชม./สัปดาห์)	<1	1-2	3-4	5-6	7-8	>8
	<input type="checkbox"/> วิ่ง <input type="checkbox"/> ว่ายน้ำ <input type="checkbox"/> ขี่จักรยาน <input type="checkbox"/> กระโดดเชือก <input type="checkbox"/> ฟันดาบ <input type="checkbox"/> ปิงปอง <input type="checkbox"/> แบดมินตัน <input type="checkbox"/> เทนนิส <input type="checkbox"/> แอร์บอล <input type="checkbox"/> แสนด์บอล <input type="checkbox"/> ฟุตบอล <input type="checkbox"/> บาสเกตบอล <input type="checkbox"/> วอลเลย์บอล <input type="checkbox"/> รักบี้ <input type="checkbox"/> ฮอกกี้ <input type="checkbox"/> เต้นแอโรบิค <input type="checkbox"/> ชีมนาสติก <input type="checkbox"/> ยกน้ำหนัก <input type="checkbox"/> อื่น ๆ ระบุ.....						

8. นักเรียนเคยแข่งกีฬาหรือเป็นนักกีฬาของโรงเรียนหรือไม่

- ( ) เคยแต่เลิกแล้ว                      ( ) เคยและยังเป็นผู้                      ( ) ไม่เคย

ถ้าเคย ได้เข้าร่วมแข่งขันกีฬาประเภทใดบ้าง (ตอบได้มากกว่า 1 ข้อ)

- ( ) กรีฑา ระบุ.....                      ( ) ปิงปอง                      ( ) แคร่บอล  
 ( ) วอลเลย์บอล                      ( ) แบดมินตัน                      ( ) วอลเลย์บอล  
 ( ) ขี่จักรยาน                      ( ) เทนนิส                      ( ) บาสเกตบอล  
 ( ) ฟุตบอล                      ( ) รักบี้                      ( ) อื่น ๆ ระบุ.....

โดยเฉลี่ยเข้าร่วมแข่งขันรวม..... ครั้ง/ปี

ระยะเวลาที่เป็นนักกีฬา..... ปี

9. ขอให้นักเรียนบอกกิจกรรมในเวลาวางที่ทำบ่อยที่สุด 4 อย่าง

1. .... ใช้เวลาโดยประมาณ..... ชั่วโมง/สัปดาห์  
 2. .... ใช้เวลาโดยประมาณ..... ชั่วโมง/สัปดาห์  
 3. .... ใช้เวลาโดยประมาณ..... ชั่วโมง/สัปดาห์  
 4. .... ใช้เวลาโดยประมาณ..... ชั่วโมง/สัปดาห์

10. เวลาที่ใช้ในการดูทีวี โดยเฉลี่ยในแต่ละวัน (ให้กาเครื่องหมายในช่องที่ต้องการ)

เวลาที่ใช้ ต่อวัน						
	ไม่ดู	น้อยกว่า 1 ชม.	1-2 ชม.	3-4 ชม.	5-6 ชม.	อื่น ๆ ระบุ
วัน						
วันปกติ						
วันหยุด						

11. นักเรียนได้เล่นวิดีโอเกมหรือไม่ ( ) ไม่เล่น ( ) เล่น

ถ้าเล่น จำนวนครั้งโดยเฉลี่ยที่นักเรียนเล่นวิดีโอเกม..... ครั้ง/สัปดาห์

ใช้เวลาในการเล่นโดยเฉลี่ยในแต่ละวันเท่าไร (ให้กาเครื่องหมายในช่องที่ต้องการ)

เวลาที่ใช้ ต่อวัน						
	ไม่เล่น	น้อยกว่า 1 ชม.	1-2 ชม.	3-4 ชม.	5-6 ชม.	อื่น ๆ ระบุ
วัน						
วันปกติ						
วันหยุด						

12. กิจกรรมในการช่วยงานบ้าน ( ) ไม่ทำ ( ) ทำ

ถ้าทำ ได้แก่

- ( ) ซักผ้า/รีดผ้า                      ใช้เวลาประมาณ..... นาที/ครั้ง จำนวน..... ครั้ง/สัปดาห์  
 ( ) กวาดบ้าน/ถูบ้าน                      ใช้เวลาประมาณ..... นาที/ครั้ง จำนวน..... ครั้ง/สัปดาห์  
 ( ) ทำกับข้าว/ล้างจาน                      ใช้เวลาประมาณ..... นาที/ครั้ง จำนวน..... ครั้ง/สัปดาห์  
 ( ) รดน้ำต้นไม้                      ใช้เวลาประมาณ..... นาที/ครั้ง จำนวน..... ครั้ง/สัปดาห์  
 ( ) อื่น ๆ ระบุ.....                      ใช้เวลาประมาณ..... นาที/ครั้ง จำนวน..... ครั้ง/สัปดาห์

13. นักเรียน เรียนพิเศษหรือไม่ ( ) ไม่เรียน ( ) เรียน  
 ถ้าเรียน จำนวนครั้งที่นักเรียนเรียนพิเศษ วันธรรมดา.....ครั้ง/สัปดาห์  
 วันหยุด.....ครั้ง/สัปดาห์  
 นักเรียนใช้เวลาในการเรียนพิเศษแต่ละครั้งเท่าไร (ให้กาเครื่องหมายในช่องที่ต้องการ)

เวลาที่ใช้ ต่อวัน	วัน					
	1 ชม.	2 ชม.	3 ชม.	4 ชม.	5 ชม.	อื่น ๆ ระบุ
วันปกติ						
วันหยุด						

14. เวลาที่ใช้ในการนอนโดยเฉลี่ย.....ชม./วัน

\_\_\_\_\_

## APPENDIX D

### Physical education class at school running

- Jumping
- Fencing
- Table Tennis
- Badminton
- Tennis
- Chairball
- Handball
- Football
- Basketball
- Volleyball
- Rugby
- Hooky
- Aerobic dancing
- Gymnastic
- Weight lifting

## APPENDIX E

Table E.1 Mean  $\pm$  SD of bone size of the subjects classified by sex and pubertal stage.

Bone size	Sex	Pubertal stage		
		Pre (79)	Early (140)	Late (55) <sup>#</sup>
Midshaft radius width	M	0.98 $\pm$ 0.09 <sup>a</sup> (56)	1.04 $\pm$ 0.08 <sup>b</sup> (56)	1.07 $\pm$ 0.11 <sup>b</sup> (25)
	F	0.94 $\pm$ 0.17 <sup>a</sup> (23)	0.97 $\pm$ 0.10 <sup>a</sup> (84)	1.02 $\pm$ 0.09 <sup>b</sup> (30)
Midshaft radius area	M	1.96 $\pm$ 0.17 <sup>a</sup>	2.09 $\pm$ 0.16 <sup>b</sup>	2.13 $\pm$ 0.22 <sup>b</sup>
	F	1.89 $\pm$ 0.16 <sup>a</sup>	1.93 $\pm$ 0.19 <sup>a</sup>	2.03 $\pm$ 0.19 <sup>b</sup>
Lumbar spine width	M	3.14 $\pm$ 0.25 <sup>a</sup>	3.36 $\pm$ 0.23 <sup>b</sup>	3.78 $\pm$ 0.29 <sup>c</sup>
	F	3.07 $\pm$ 0.21 <sup>a</sup>	3.28 $\pm$ 0.27 <sup>b</sup>	3.58 $\pm$ 0.33 <sup>c</sup>
Lumbar spine height	M	7.71 $\pm$ 0.44 <sup>a</sup>	8.06 $\pm$ 0.44 <sup>b</sup>	8.88 $\pm$ 0.52 <sup>c</sup>
	F	7.75 $\pm$ 0.28 <sup>a</sup>	8.39 $\pm$ 0.57 <sup>b</sup>	8.92 $\pm$ 0.47 <sup>c</sup>
Femoral neck width	M	24.0 $\pm$ 1.7 <sup>a</sup>	25.3 $\pm$ 1.9 <sup>b</sup>	27.4 $\pm$ 2.3 <sup>c</sup>
	F	22.7 $\pm$ 1.4 <sup>a</sup>	24.5 $\pm$ 2.3 <sup>b</sup>	25.0 $\pm$ 2.3 <sup>b</sup>
Femoral neck height	M	14.5 $\pm$ 1.2 <sup>a</sup>	14.5 $\pm$ 1.1 <sup>a</sup>	15.1 $\pm$ 0.7 <sup>b</sup>
	F	13.9 $\pm$ 1.6 <sup>a</sup>	14.3 $\pm$ 1.4 <sup>a, b</sup>	14.9 $\pm$ 0.5 <sup>b</sup>
Femoral neck volume	M	6.61 $\pm$ 1.15 <sup>a</sup>	7.32 $\pm$ 1.29 <sup>b</sup>	9.01 $\pm$ 1.96 <sup>c</sup>
	F	5.68 $\pm$ 1.08 <sup>a</sup>	6.83 $\pm$ 1.48 <sup>b, c</sup>	7.39 $\pm$ 1.44 <sup>c</sup>
Lumbar spine volume	M	58.5 $\pm$ 16.9 <sup>a</sup>	69.5 $\pm$ 18.6 <sup>b</sup>	96.8 $\pm$ 27.7 <sup>c</sup>
	F	57.5 $\pm$ 8.7 <sup>a</sup>	70.8 $\pm$ 16.9 <sup>b</sup>	87.9 $\pm$ 25.4 <sup>c</sup>

<sup>#</sup> Figures in the parentheses are number of subjects

<sup>a, b, c</sup> Mean within the same row with different superscript are significant difference ( $p < 0.05$ )

Table E.2 Mean ± SD of bone size of the subjects classified by sex and age group

Bone size	Sex	Age group				Total
		9.00-9.99	10.00-10.99	11.00-11.99	12.00-12.99	
Midshaft radius width	M	1.00 ± 0.09 <sup>a</sup> (29)	1.00 ± 0.07 <sup>ab</sup> (35)	1.04 ± 0.09 <sup>ab</sup> (39)	1.06 ± 0.10 <sup>b</sup> (34)	1.02 ± 0.09 (137) <sup>#</sup>
	F	0.94 ± 0.09 <sup>a</sup> (27)	0.95 ± 0.08 <sup>a,*</sup> (38)	0.98 ± 0.09 <sup>ab,*</sup> (37)	1.02 ± 0.10 <sup>b</sup> (35)	0.98 ± 0.09 (137)
Midshaft radius area	M	1.96 ± 0.18 <sup>a</sup>	2.01 ± 0.14 <sup>ab</sup>	2.08 ± 0.19 <sup>ab</sup>	2.12 ± 0.20 <sup>b</sup>	2.05 ± 0.19
	F	1.9 ± 0.19 <sup>a</sup>	1.90 ± 0.15 <sup>a,*</sup>	1.96 ± 0.18 <sup>ab,*</sup>	2.03 ± 0.22 <sup>b</sup>	1.95 ± 0.19
Lumbar spine width	M	3.09 ± 0.20 <sup>a</sup>	3.20 ± 0.26 <sup>a</sup>	3.36 ± 0.23 <sup>b</sup>	3.70 ± 0.31 <sup>c</sup>	3.35 ± 0.34
	F	3.13 ± 0.30 <sup>a</sup>	3.21 ± 0.29 <sup>a</sup>	3.32 ± 0.27 <sup>a</sup>	3.53 ± 0.31 <sup>b</sup>	3.31 ± 0.32
Lumbar spine height	M	7.57 ± 0.39 <sup>a</sup>	7.80 ± 0.35 <sup>ab</sup>	8.09 ± 0.46 <sup>b</sup>	8.72 ± 0.54 <sup>c</sup>	8.07 ± 0.61
	F	7.89 ± 0.41 <sup>a,*</sup>	8.07 ± 0.49 <sup>a,*</sup>	8.58 ± 0.47 <sup>b,*</sup>	8.94 ± 0.50 <sup>c</sup>	8.39 ± 0.62
Femoral neck width	M	23.6 ± 1.4 <sup>a</sup>	24.4 ± 1.4 <sup>ab</sup>	25.4 ± 2.1 <sup>b</sup>	26.9 ± 2.4 <sup>c</sup>	25.1 ± 2.3
	F	23.3 ± 1.9 <sup>a</sup>	23.7 ± 2.1 <sup>a</sup>	24.4 ± 1.7 <sup>ab,*</sup>	25.7 ± 2.6 <sup>b,*</sup>	24.3 ± 2.3
Femoral neck height	M	14.6 ± 1.2	14.4 ± 1.2 <sup>a</sup>	14.5 ± 1.1 <sup>ab</sup>	15.1 ± 0.6 <sup>b</sup>	14.6 ± 1.1
	F	14.0 ± 1.6	13.6 ± 1.6 <sup>a,*</sup>	14.8 ± 0.7 <sup>b</sup>	14.9 ± 0.4 <sup>c</sup>	14.4 ± 1.3
Femoral neck volume	M	6.37 ± 0.94 <sup>a</sup>	6.75 ± 1.03 <sup>ab</sup>	7.39 ± 1.39 <sup>b</sup>	8.70 ± 1.89 <sup>c</sup>	7.33 ± 1.61
	F	5.97 ± 1.04 <sup>a</sup>	6.10 ± 1.51 <sup>a</sup>	6.99 ± 1.06 <sup>b</sup>	7.84 ± 1.52 <sup>b</sup>	6.76 ± 1.50
lumbar spine volume	M	57.2 ± 9.61 <sup>a</sup>	58.0 ± 21.2 <sup>a</sup>	68.3 ± 20.0 <sup>a</sup>	94.9 ± 20.8 <sup>b</sup>	69.9 ± 24.0
	F	61.4 ± 12.1 <sup>a</sup>	66.5 ± 15.1 <sup>a</sup>	71.2 ± 23.1 <sup>a</sup>	88.4 ± 18.6 <sup>b</sup>	72.4 ± 20.4

<sup>#</sup> Figures in the parentheses are number of subjects

**Table E. 3** Correlation coefficient between various variables and bone parameter of male subjects

Variable	WAZ	HAZ	Calcium intake	WBA	Midshaft radius BMD	L2-4 BMD	FNBMID	L2-4V	FNVBMD	Total BMD	Midshaft radius BMC	L2-4 BMC	FNBMC	TOTAL BMC	TBC
Age	0.086	0.156	0.013	0.150	0.491**	0.444**	0.172*	-0.183*	-0.151	0.292**	0.606**	0.668**	0.359**	0.694**	0.694**
WAZ		0.733**	0.172*	0.006	0.323**	0.434**	0.334**	-0.014	0.002	0.458**	0.503**	0.525**	0.325**	0.596**	0.597**
HAZ			0.237**	0.022	0.373**	0.385**	0.272**	-0.145	-0.041	0.374**	0.455**	0.574**	0.363**	0.608**	0.607**
Calcium intake				0.139	0.199*	0.060	0.163	-0.118	0.014	0.165	0.154	0.111	0.178*	0.157	0.157
WBA					0.167	0.295**	0.199	0.041	0.077	0.315**	0.188*	0.268**	0.213**	0.262**	0.261**
Midshaft radius BMD						0.562**	0.375**	0.066	0.002	0.665**	0.772**	0.646**	0.436**	0.718**	0.718**
L2-4 BMD							0.589**	0.565**	0.201*	0.785**	0.646**	0.851**	0.527**	0.788**	0.789**
FN BMD								0.296**	0.497**	0.592**	0.419**	0.504**	0.649**	0.535**	0.534**
L2-4V									0.362**	0.357**	-0.003	0.086	0.138	0.048	0.048
FNVBMD										0.141	-0.049	0.019	0.546**	0.010	0.010
Total BMD											0.633**	0.691**	0.451**	0.782**	0.782**
Midshaft radius BMC												0.769**	0.479**	0.829**	0.830**
L2-4 BMC													0.599**	0.932**	0.932**
FN BMC														0.599**	0.599**
TOTAL BMC															1.000**

\*, \*\* significant at p<0.05, p<0.01 respectively

Table E.4 Correlation coefficient between various variables and bone parameter of female subjects

Variable	WAZ	HAZ	Calcium Intake	WBA	Midshaft radius BMD	L2-4 BMD	FNBMID	L2-4V	FNVBMD	Total BMD	Midshaft radius BMC	L2-4 BMC	FNBMC	TOTAL BMC	TBC
Age	-0.044	-0.113	0.013	0.077	0.570**	0.627**	0.406**	0.398**	0.068	0.395**	0.613**	0.659**	0.568**	0.679**	0.680**
WAZ		0.622**	0.135	-0.030	0.303**	0.386**	0.390**	-0.056	0.124	0.451**	0.336**	0.462**	0.442**	0.558**	0.558**
HAZ			0.122	0.101	0.167	0.213*	0.216*	-0.215*	0.008	0.160	0.173*	0.378**	0.337**	0.370**	0.369**
Calcium Intake				0.200*	0.162	0.118	0.119	0.062	0.096	0.125	0.207*	0.100	0.146	0.135	0.135
WBA					-0.103	-0.015	-0.043	-0.022	-0.098	-0.105	-0.029	-0.024	-0.043	-0.005	-0.005
Midshaft radius BMD						0.736**	0.592**	0.419**	0.213*	0.747**	0.813**	0.741**	0.652**	0.775**	0.775**
L2-4 BMD							0.677**	0.667**	0.328**	0.781**	0.746**	0.917**	0.740**	0.867**	0.867**
FN BMD								0.330**	0.707**	0.739**	0.600**	0.674**	0.828**	0.727**	0.728**
L2-4V									0.197*	0.479**	0.382**	0.356**	0.321**	0.364**	0.364**
FNVBMD										0.474**	0.138	0.251**	0.405**	0.285**	0.286**
Total BMD											0.671**	0.732**	0.664**	0.824**	0.824**
Midshaft radius BMC												0.781**	0.706**	0.810**	0.810**
L2-4 BMC													0.789**	0.927**	0.927**
FN BMC														0.821**	0.821**
TOTAL BMC															1.000**

\*, \*\* significant at P<0.05, P<0.01 respectively

## APPENDIX F

**Table F1** Mean  $\pm$  SD of BMD at lumbar spine and femoral neck of the subjects comparing with young adult<sup>(127)</sup>

Bone mineral density	Sex	Age group (y)	
		9-12	20-29
Lumbar spine	M	0.74 $\pm$ 0.09 (65%)	1.13 $\pm$ 0.1
	F	0.85 $\pm$ 0.11 (73%)	1.16 $\pm$ 0.1
Femoral neck	M	0.80 $\pm$ 0.11 (78%)	1.02 $\pm$ 0.1
	F	0.78 $\pm$ 0.10 (90%)	0.87 $\pm$ 0.1

**Table F2** Mean  $\pm$  SD of total body bone mass of Thai children comparing with reference data<sup>(Maynard 1998)</sup>

Total body bone mass	Sex	Age group				
		9.00 - 9.99	10.00 - 10.99	11.00 - 11.99	12.00 - 12.99	
Total BMD	M	0.88 $\pm$ 0.04 (29)	0.88 $\pm$ 0.05 (35)	0.90 $\pm$ 0.05 (39)	0.93 $\pm$ 0.08 (34)	
	Caucasian	0.90 $\pm$ 0.05 (10)	0.92 $\pm$ 0.04** (16)	0.94 $\pm$ 0.06** (24)	0.91 $\pm$ 0.05 (29)	
Total BMC	F	0.89 $\pm$ 0.06 (27)	0.91 $\pm$ 0.05 (38)	0.93 $\pm$ 0.07 (37)	0.96 $\pm$ 0.07 (35)	
	Caucasian	0.89 $\pm$ 0.05 (11)	0.90 $\pm$ 0.06 (16)	0.93 $\pm$ 0.07 (24)	0.99 $\pm$ 0.09 (24)	
Total BMC	M	1098.6 $\pm$ 116.0	1212.4 $\pm$ 160.3	1353.7 $\pm$ 211.7	1645.1 $\pm$ 288.2	
	Caucasian	1215 $\pm$ 108*	1297 $\pm$ 126	1447 $\pm$ 222	1541 $\pm$ 251	
Total BMC	F	1164.3 $\pm$ 200.5	1275.2 $\pm$ 181.2	1473.6 $\pm$ 233.6	1679.5 $\pm$ 245.3	
	Caucasian	1167 $\pm$ 122	1244 $\pm$ 147	1438 $\pm$ 235	1673 $\pm$ 325	

\*, \*\* significant difference at P<0.05, P< 0.01 respectively

## APPENDIX G

Table G.1 Multiple regression analysis of various factors on midshaft radius BMD

Variable	b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equation
<b>Model 1 : Physiological factors</b>						
Age	0.025	0.004	0.438	0.000	0.406	0.050
Sex	0.015	0.006	0.123	0.015		
Weight/age	0.014	0.005	0.190	0.004		
Height/age	0.007	0.005	0.087	0.181		
Pre-puberty	-0.026	0.012	-0.201	0.025		
Early-puberty	-0.031	0.008	-0.260	0.000		
Constant	0.237	0.045	-	0.000		
<b>Model 2 : Environmental factors</b>						
2.1 Usual calcium intake	0.0004	0.001	0.186	0.003	0.026	0.06
Weight bearing activity	-0.0004	0.001	-0.37	0.544		
Constant	0.474	0.009	-	0.000		
2.2 Current calcium intake	0.000013	0.000	0.049	0.419	-0.005	0.060
Weight bearing activity	0.0000091	0.001	0.001	0.988		
Constant	0.490	0.010	-	0.000		
2.3 Absolute value						
Current calcium intake	0.00000057	0.000	0.002	0.975	-0.003	0.060
Protein	0.00012	0.000	0.040	0.754		
Phosphorus	0.000020	0.000	0.073	0.534		
Sodium	0.00000001	0.000	0.002	0.979		
Constant	0.475	0.014	-	0.000		
2.4 Nutrient density						
Current calcium intake	-0.000019	0.000	-0.039	0.510	0.052	0.060
Protein	-0.025	0.006	-0.255	0.000		
Phosphorus	0.000035	0.000	0.051	0.433		
Sodium	-0.0000072	0.000	-0.063	0.323		
Constant	0.548	0.022	-	0.000		

**Table G.1 (cont.)**

Variable	b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equation		
<b>Model 3 : All factors</b>								
3.1 Age	0.025	0.004	0.445	0.000	0.414	0.050		
Sex	0.012	0.006	0.099	0.063				
Weight/age	0.013	0.005	0.182	0.005				
Height/age	0.006	0.005	0.076	0.249				
Pre-puberty	-0.026	0.012	-0.196	0.029				
Early-puberty	-0.029	0.008	-0.244	0.001				
Usual calcium intake	0.00002	0.000	0.122	0.013				
Weight bearing activity	-0.0006	0.001	-0.059	0.252				
Constant	0.220	0.046	-	0.000				
3.2 Age	0.027	0.004	0.471	0.000			0.339	0.046
Sex	0.015	0.006	0.130	0.014				
Weight/age	0.015	0.005	0.198	0.004				
Height/age	0.007	0.005	0.093	0.164				
Pre-puberty	-0.025	0.012	-0.192	0.035				
Early-puberty	-0.030	0.008	-0.256	0.000				
Current calcium intake	0.000028	0.000	0.058	0.241				
Protein	-0.0043	0.005	-0.044	0.431				
Phosphorus	-0.000018	0.000	-0.026	0.620				
Sodium	0.00000018	0.000	0.002	0.976				
Constant	0.205	0.055	-	0.000				



**Table G.2** Multiple regression analysis of various factors on lumbar spine BMD

Variable	b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equation
<b>Model 1 : Physiological factors</b>						
Age	0.039	0.005	0.365	0.000	0.613	0.069
Sex	0.107	0.009	0.477	0.000		
Weight/age	0.039	0.007	0.282	0.000		
Height/age	0.0006	0.008	0.004	0.938		
Pre-puberty	-0.065	0.018	-0.262	0.000		
Early-puberty	-0.075	0.013	-0.333	0.000		
Constant	0.366	0.069	-	0.000		
<b>Model 2 : Environmental factors</b>						
2.1 Usual calcium intake	0.00004	0.000	0.102	0.101	0.005	0.112
Weight bearing activity	-0.0013	0.001	-0.70	0.261		
Constant	0.776	0.018	-	0.000		
2.2 Current calcium intake	-0.000003	0.000	-0.006	0.919	-0.005	0.112
Weight bearing activity	0.000009	0.001	0.001	0.988		
Constant	0.490	0.010	-	0.000		
2.3 Absolute value						
Current calcium intake	-0.000023	0.000	-0.048	0.501	-0.009	0.113
Protein	0.00072	0.001	0.128	0.318		
Phosphorus	-0.000046	0.000	-0.089	0.454		
Sodium	0.0000033	0.000	0.028	0.709		
Constant	0.777	0.026	-	0.000		
2.4 Nutrient density						
Current calcium intake	-0.0000158	0.000	-0.017	0.770	0.092	0.107
Protein	-0.06142	0.011	-0.330	0.000		
Phosphorus	0.000064	0.000	0.049	0.443		
Sodium	-0.0000026	0.000	-0.012	0.846		
Constant	0.893	0.040	-	0.000		

**Table G.2 (cont.)**

<b>Variable</b>		<b>b</b>	<b>SEb</b>	<b>BETA</b>	<b>p value</b>	<b>Adjusted R<sup>2</sup></b>	<b>SE of Equation</b>
<b>Model 3 : All factors</b>							
3.1	Age	0.037	0.006	0.349	0.000	0.613	0.07
	Sex	0.113	0.010	0.506	0.000		
	Weight/age	0.040	0.007	0.287	0.000		
	Height/age	-0.0007	0.008	-0.005	0.925		
	Pre-puberty	-0.068	0.018	-0.275	0.000		
	Early-puberty	-0.076	0.013	-0.341	0.000		
	Usual calcium intake	-0.000005	0.000	-0.014	0.733		
	Weight bearing activity	0.0015	0.001	0.079	0.062		
	Constant	0.377	0.070	-	0.000		
3.2	Age	0.042	0.006	0.393	0.000		
	Sex	0.108	0.009	0.481	0.000		
	Weight/age	0.040	0.008	0.289	0.000		
	Height/age	0.0014	0.008	0.010	0.859		
	Pre-puberty	-0.063	0.018	-0.254	0.001		
	Early-puberty	-0.0748	0.013	-0.334	0.000		
	Current calcium intake	0.000035	0.000	0.037	0.347		
	Protein	0.0060	0.008	0.032	0.476		
	Phosphorus	-0.000037	0.000	-0.028	0.502		
	Sodium	0.0000056	0.000	0.026	0.532		
	Constant	0.315	0.083	-	0.000		

**Table G.3** Multiple regression analysis of various factors on femoral neck BMD

Variable	b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equation
<b>Model 1 : Physiological factors</b>						
Age	0.023	0.007	0.233	0.001	0.234	0.089
Sex	-0.002	0.012	-0.012	0.839		
Weight/age	0.040	0.009	0.313	0.000		
Height/age	0.00009	0.010	0.001	0.992		
Pre-puberty	-0.031	0.023	-0.136	0.178		
Early-puberty	-0.057	0.016	-0.278	0.001		
Constant	0.571	0.089	-	0.000		
<b>Model 2 : Environmental factors</b>						
2.1 Usual calcium intake	0.00004	0.000	0.123	0.047	0.020	0.101
Weight bearing activity	0.0015	0.001	0.086	0.163		
Constant	0.743	0.016	-	0.000		
2.2 Current calcium intake	0.000051	0.000	0.114	0.060	0.019	0.101
Weight bearing activity	0.0019	0.001	0.107	0.077		
Constant	0.744	0.016	-	0.000		
2.3 Absolute value						
Current calcium intake	0.000028	0.000	0.063	0.372	-0.009	0.113
Protein	0.00115	0.001	0.227	0.073		
Phosphorus	-0.000058	0.000	-0.121	0.300		
Sodium	-0.0000057	0.000	-0.053	0.479		
Constant	0.741	0.023	-	0.000		
2.4 Nutrient density						
Current calcium intake	0.0000356	0.000	0.042	0.490	0.019	0.102
Protein	-0.027	0.011	-0.159	0.012		
Phosphorus	0.0000141	0.000	0.001	0.986		
Sodium	-0.000019	0.000	-0.096	0.140		
Constant	0.853	0.038	-	0.000		

**Table G.3 (cont.)**

Variable	b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equation
<b>Model 3 : All factors</b>						
3.1 Age	0.021	0.007	0.219	0.003	0.234	0.089
Sex	0.002	0.012	0.010	0.865		
Weight/age	0.039	0.009	0.313	0.000		
Height/age	-0.0018	0.010	-0.014	0.856		
Pre-puberty	-0.033	0.023	-0.146	0.153		
Early-puberty	-0.057	0.016	-0.278	0.001		
Usual calcium intake	0.000018	0.000	0.050	0.373		
Weight bearing activity	0.001	0.001	0.061	0.305		
Constant	0.566	0.090	-	0.000		
3.2 Age	0.028	0.008	0.288	0.000		
Sex	0.0012	0.012	0.006	0.919		
Weight/age	0.042	0.010	0.333	0.000		
Height/age	0.0016	0.010	0.012	0.875		
Pre-puberty	-0.0229	0.023	-0.101	0.320		
Early-puberty	-0.053	0.016	-0.261	0.001		
Current calcium intake	0.000073	0.000	0.086	0.120		
Protein	0.0093	0.011	0.055	0.383		
Phosphorus	-0.000077	0.000	-0.064	0.276		
Sodium	-0.0000101	0.000	-0.051	0.374		
Constant	0.512	0.105	-	0.000		

**Table G.4** Multiple regression analysis of various factors on total body BMD

Variable	b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equation
<b>Model 1 : Physiological factors</b>						
Age	0.017	0.004	0.274	0.000	0.366	0.05
Sex	0.029	0.007	0.233	0.000		
Weight/age	0.036	0.005	0.452	0.000		
Height/age	-0.007	0.006	-0.085	0.209		
Pre-puberty	-0.022	0.013	-0.152	0.098		
Early-puberty	-0.032	0.009	-0.251	0.001		
Constant	0.735	0.050	-	0.000		
<b>Model 2: Environmental factors</b>						
2.1 Usual calcium intake	0.00003	0.000	0.138	0.026	0.013	0.06
Weight bearing	0.00023	0.001	0.021	0.738		
Constant	0.889	0.010	-	0.000		
2.2 Current calcium intake	0.000034	0.000	0.122	0.045	0.010	0.06
Weight bearing	0.00052	0.001	0.047	0.438		
Constant	0.890	0.010	-	0.000		
2.3 Absolute value						
Current calcium intake	0.000022	0.000	0.078	0.270	0.009	0.064
Protein	0.00059	0.000	0.183	0.147		
Phosphorus	-0.000034	0.000	-0.113	0.331		
Sodium	-0.0000016	0.000	-0.024	0.751		
Constant	0.885	0.015	-	0.000		
2.4 Nutrient density						
Current calcium intake	0.000039	0.000	0.074	0.214	0.046	0.063
Protein	-0.026	0.007	-0.246	0.000		
Phosphorus	0.000019	0.000	0.026	0.696		
Sodium	-0.0000054	0.000	-0.043	0.495		
Constant	0.948	0.024	-	0.000		

**Table G.4 (cont.)**

Variable		b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equation
<b>Model 3 : All factors</b>							
3.1	Age	0.015	0.004	0.250	0.000	0.368	0.05
	Sex	0.033	0.007	0.258	0.000		
	Weight/age	0.036	0.005	0.455	0.000		
	Height/age	-0.009	0.006	-0.106	0.122		
	Pre-puberty	-0.024	0.013	-0.172	0.064		
	Early-puberty	-0.033	0.009	-0.254	0.001		
	Usual calcium intake	0.00001	0.000	0.048	0.341		
	Weight bearing activity	0.00090	0.001	0.082	0.131		
	Constant	0.738	0.051	-	0.000		
3.2	Age	0.0203	0.004	0.334	0.000		
	Sex	0.0313	0.007	0.244	0.000		
	Weight/age	0.0374	0.006	0.468	0.000		
	Height/age	-0.0062	0.006	-0.075	0.269		
	Pre-puberty	-0.019	0.013	-0.132	0.156		
	Early-puberty	-0.0311	0.009	-0.242	0.001		
	Current calcium intake	0.000057	0.000	0.108	0.032		
	Protein	0.00722	0.006	0.069	0.233		
	Phosphorus	-0.000038	0.000	-0.051	0.340		
	Sodium	-0.0000016	0.000	-0.013	0.801		
	Constant	0.679	0.060	-	0.000		

**Table G.5** Multiple regression analysis of various factors on lumbar spine vBMD

Variable	b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equation
<b>Model 1 : Physiological factors</b>						
Age	0.0009	0.002	0.026	0.688	0.414	0.027
Sex	0.045	0.004	0.641	0.000		
Weight/age	0.0042	0.003	0.095	0.148		
Height/age	-0.011	0.003	-0.241	0.000		
Pre-puberty	-0.013	0.007	-0.172	0.055		
Early-puberty	-0.015	0.005	-0.217	0.002		
Constant	0.285	0.027	-	0.000		
<b>Model 2 : Environmental factors</b>						
2.1 Usual calcium intake	0.000004	0.000	0.032	0.604	0.037	0.035
Weight bearing activity	-0.0013	0.000	-0.214	0.001		
Constant	0.310	0.005	-	0.000		
2.2 Current calcium intake	-0.0000013	0.000	-0.009	0.885	0.034	0.035
Weight bearing activity	-0.00122	0.000	-0.203	0.001		
Constant	0.313	0.006	-	0.000		
2.3 Absolute value						
Current calcium intake	-0.0000024	0.000	-0.016	0.825	0.001	0.035
Protein	0.00027	0.000	0.158	0.221		
Phosphorus	-0.000038	0.000	-0.233	0.050		
Sodium	0.00000064	0.000	0.018	0.819		
Constant	0.309	0.008	-	0.000		
2.4 Nutrient density						
Current calcium intake	0.000013	0.000	0.045	0.468	-0.004	0.035
Protein	-0.0030	0.004	-0.052	0.418		
Phosphorus	-0.000013	0.000	-0.031	0.649		
Sodium	0.0000052	0.000	0.077	0.243		
Constant	0.302	0.013	-	0.000		

**Table G.5 (cont.)**

Variable	b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equation		
<b>Model 3 : All factors</b>								
3.1 Age	0.00005	0.002	0.015	0.824	0.408	0.027		
Sex	0.046	0.004	0.648	0.000				
Weight/age	0.004	0.003	0.097	0.141				
Height/age	-0.011	0.003	-0.246	0.000				
Pre-puberty	-0.014	0.007	-0.183	0.045				
Early-puberty	-0.016	0.005	-0.222	0.002				
Usual calcium intake	0.00017	0.000	0.028	0.593				
Weight bearing activity	-0.0000015	0.000	-0.013	0.799				
Constant	0.289	0.027	-	0.000				
3.2 Age	-0.0025	0.002	0.076	0.271			0.419	0.0269
Sex	0.0465	0.004	0.661	0.000				
Weight/age	0.0059	0.003	0.134	0.052				
Height/age	-0.011	0.003	-0.251	0.000				
Pre-puberty	-0.013	0.007	-0.174	0.055				
Early-puberty	-0.016	0.005	-0.224	0.002				
Current calcium intake	0.0000052	0.000	0.018	0.714				
Protein	0.00749	0.003	0.131	0.020				
Phosphorus	-0.000017	0.000	-0.041	0.434				
Sodium	-0.0000003	0.000	0.047	0.358				
Constant	0.251	0.032	-	0.000				

**Table G.6** Multiple regression analysis of various factors on femoral neck vBMD

Variable	b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equation
<b>Model 1 : Physiological factors</b>						
Age	-0.007	0.007	-0.089	0.276	0.034	0.09
Sex	0.02	0.011	0.116	0.071		
Weight/age	0.01	0.009	0.091	0.280		
Height/age	-0.01	0.010	-0.103	0.222		
Pre-puberty	-0.03	0.022	-0.135	0.231		
Early-puberty	-0.05	0.016	-0.260	0.004		
Constant	0.538	0.086	-	0.000		
<b>Model 2 : Environmental factors</b>						
2.1 Usual calcium intake	0.000019	0.000	0.063	0.312	-0.003	0.088
Weight bearing activity	-0.00057	0.001	-0.038	0.547		
Constant	0.424	0.014	-	0.000		
2.2 Current calcium intake	0.000029	0.000	0.076	0.217	-0.001	0.088
Weight bearing activity	-0.00040	0.001	-0.027	0.665		
Constant	0.420	0.014	-	0.000		
2.3 Absolute value						
Current calcium intake	0.000031	0.000	0.080	0.262	0.006	0.09
Protein	0.00058	0.001	0.132	0.302		
Phosphorus	-0.000047	0.000	-0.114	0.335		
Sodium	-0.000011	0.000	-0.116	0.129		
Constant	0.436	0.020	-	0.000		
2.4 Nutrient density						
Current calcium intake	0.000079	0.000	0.107	0.083	0.002	0.09
Protein	-0.0032	0.009	-0.022	0.730		
Phosphorus	0.0000049	0.000	0.005	0.944		
Sodium	-0.000013	0.000	-0.076	0.248		
Constant	0.434	0.033	-	0.000		

**Table G.6 (cont.)**

Variable	b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equation
<b>Model 3 : All factors</b>						
3.1 Age	-0.008	0.007	-0.099	0.237	0.028	0.009
Sex	0.021	0.012	0.120	0.083		
Weight/age	0.0099	0.009	0.090	0.289		
Height/age	-0.013	0.010	-0.112	0.192		
Pre-puberty	-0.028	0.022	-0.144	0.208		
Early-puberty	-0.045	0.016	-0.259	0.005		
Usual calcium intake	0.00001	0.000	0.034	0.591		
Weight bearing activity	0.00029	0.001	0.019	0.774		
Constant	0.538	0.088	-	0.000		
3.2 Age	-0.0054	0.007	-0.064	0.463		
Sex	0.0209	0.012	0.119	0.076		
Weight/age	0.0102	0.010	0.092	0.298		
Height/age	-0.011	0.010	-0.097	0.259		
Pre-puberty	-0.0235	0.022	-0.120	0.296		
Early-puberty	-0.0436	0.016	-0.248	0.007		
Current calcium intake	0.000060	0.000	0.082	0.193		
Protein	0.00173	0.010	0.012	0.869		
Phosphorus	0.00015	0.000	0.002	0.982		
Sodium	-0.000013	0.000	-0.076	0.247		
Constant	0.512	0.102	-	0.000		

**Table G.7** Multiple regression analysis of various factors on midshaft radius BMC

Variable	b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equation
<b>Model 1 : Physiological factors</b>						
Age	0.076	0.008	0.518	0.000	0.542	0.11
Sex	-0.019	0.014	-0.060	0.171		
Weight/age	0.065	0.011	0.338	0.000		
Height/age	0.0056	0.011	0.028	0.622		
Pre-puberty	-0.064	0.027	-0.188	0.017		
Early-puberty	-0.064	0.019	-0.207	0.001		
Constant	0.207	0.104	-	0.047		
<b>Model 2 : Environmental factors</b>						
2.1 Usual calcium intake	0.00009	0.000	0.168	0.006	0.031	0.15
Weight bearing activity	0.0019	0.002	0.072	0.238		
Constant	0.919	0.024	-	0.000		
2.2 Current calcium intake	0.000030	0.000	0.045	0.461	0.006	0.155
Weight bearing activity	0.0028	0.002	0.106	0.080		
Constant	0.958	0.025	-	0.000		
2.3 Absolute value						
Current calcium intake	-0.000023	0.000	-0.035	0.623	0.014	0.154
Protein	0.000801	0.001	0.103	0.412		
Phosphorus	0.000058	0.000	0.079	0.496		
Sodium	0.0000022	0.000	0.013	0.859		
Constant	0.906	0.035	-	0.000		
2.4 Nutrient density						
Current calcium intake	-0.000099	0.000	-0.078	0.188	0.078	0.149
Protein	-0.073	0.016	-0.287	0.000		
Phosphorus	0.0001	0.000	0.056	0.387		
Sodium	-0.000029	0.000	-0.096	0.124		
Constant	1.171	0.056	-	0.000		

**Table G.7 (cont.)**

Variable		b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equation
<b>Model 3 : All factors</b>							
3.1	Age	0.077	0.008	0.520	0.000	0.550	0.10
	Sex	-0.021	0.014	-0.069	0.141		
	Weight/age	0.064	0.011	0.332	0.000		
	Height/age	0.0029	0.011	0.015	0.798		
	Pre-puberty	-0.063	0.027	-0.184	0.019		
	Early-puberty	-0.060	0.019	-0.194	0.002		
	Usual calcium intake	0.000059	0.000	0.111	0.009		
	Weight bearing activity	-0.00062	0.001	-0.023	0.613		
	Constant	0.165	0.105	-	0.118		
3.2	Age	0.0798	0.009	0.540	0.000	0.536	0.1058
	Sex	-0.0163	0.014	-0.053	0.254		
	Weight/age	0.0673	0.012	0.348	0.000		
	Height/age	0.00632	0.012	0.032	0.589		
	Pre-puberty	-0.0605	0.027	-0.177	0.027		
	Early-puberty	-0.0619	0.019	-0.200	0.002		
	Current calcium intake	0.000044	0.000	0.034	0.429		
	Protein	0.0081	0.013	0.032	0.519		
	Phosphorus	-0.000060	0.000	-0.033	0.470		
	Sodium	-0.00006	0.000	-0.020	0.660		
	Constant	0.172	0.125	-	0.169		

**Table G.8** Multiple regression analysis of various factors on lumbar spine BMC

Variable	b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equation
<b>Model 1 : Physiological factors</b>						
Age	3.091	0.241	0.526	0.000	0.761	3.05
Sex	3.308	0.400	0.265	0.000		
Weight/age	1.946	0.323	0.251	0.000		
Height/age	1.796	0.334	0.226	0.000		
Pre-puberty	-3.295	0.784	-0.240	0.000		
Early-puberty	-4.013	0.561	-0.322	0.000		
Constant	-10.775	3.025	-	0.003		
<b>Model 2 : Environmental factors</b>						
2.1 Usual calcium intake	0.0022	0.001	0.104	0.097	0.003	6.23
Weight bearing activity	-0.0019	0.067	-0.002	0.977		
Constant	20.715	0.988	-	0.000		
2.2 Current calcium intake	-0.00116	0.002	-0.043	0.488	-0.005	6.262
Weight bearing activity	0.0259	0.066	0.024	0.694		
Constant	22.608	1.012	-	0.000		
2.3 Absolute value						
Current calcium intake	-0.00268	0.002	-0.099	0.171	0.002	6.239
Protein	0.01198	0.040	0.039	0.763		
Phosphorus	0.00198	0.003	0.068	0.564		
Sodium	0.00031	0.000	0.048	0.535		
Constant	20.643	1.435	-	0.000		
2.4 Nutrient density						
Current calcium intake	-0.0047	0.003	-0.092	0.105	0.164	5.711
Protein	-4.199	0.595	-0.413	0.000		
Phosphorus	0.0066	0.004	0.092	0.137		
Sodium	-0.00076	0.001	-0.064	0.291		
Constant	30.297	2.162	-	0.000		

**Table G.8 (cont.)**

Variable	b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equation
<b>Model 3 : All factors</b>						
3.1 Age	3.062	0.247	0.521	0.000	0.760	3.06
Sex	3.502	0.430	0.281	0.000		
Weight/age	1.967	0.324	0.254	0.000		
Height/age	1.782	0.338	0.224	0.000		
Pre-puberty	-3.339	0.794	-0.243	0.000		
Early-puberty	-4.074	0.566	-0.327	0.000		
Usual calcium intake	-0.00035	0.001	-0.017	0.594		
Weight bearing activity	0.041	0.036	0.039	0.249		
Constant	-10.538	3.098	-	0.001		
3.2 Age	3.109	0.260	0.530	0.000		
Sex	3.243	0.420	0.260	0.000		
Weight/age	1.869	0.343	0.240	0.000		
Height/age	1.865	0.340	0.234	0.000		
Pre-puberty	-3.189	0.800	-0.232	0.000		
Early-puberty	-3.996	0.569	-0.320	0.000		
Current calcium intake	0.00186	0.002	0.023	0.467		
Protein	-0.285	0.365	-0.028	0.435		
Phosphorus	-0.0011	0.002	-0.016	0.641		
Sodium	0.000059	0.000	0.005	0.880		
Constant	-10.501	3.638	-	0.004		

**Table G.9** Multiple regression analysis of various factors on femoral neck BMC

Variable	b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equation
<b>Model 1 : Physiological factors</b>						
Age	0.244	0.042	0.380	0.000	0.390	0.52
Sex	-0.153	0.068	-0.114	0.026		
Weight/age	0.169	0.056	0.203	0.003		
Height/age	0.147	0.058	0.171	0.012		
Pre-puberty	-0.243	0.134	-0.163	0.070		
Early-puberty	-0.382	0.096	-0.285	0.000		
Constant	0.629	0.521	-	0.228		
<b>Model 2 : Environmental factors</b>						
2.1 Usual calcium intake	0.00031	0.000	0.134	0.030	0.034	0.66
Weight bearing activity	0.014	0.007	0.125	0.042		
Constant	2.69	0.105	-	0.000		
2.2 Current calcium intake	0.000031	0.000	0.011	0.861	0.018	0.666
Weight bearing activity	0.018	0.007	0.159	0.010		
Constant	2.859	0.107	-	0.000		
2.3 Absolute value						
Current calcium intake	-0.00013	0.000	-0.046	0.523	-0.002	0.673
Protein	0.00535	0.004	0.160	0.212		
Phosphorus	-0.00020	0.000	-0.065	0.582		
Sodium	0.000025	0.000	0.036	0.636		
Constant	2.757	0.154	-	0.000		
2.4 Nutrient density						
Current calcium intake	-0.00033	0.000	-0.060	0.319	0.050	0.655
Protein	-0.272	0.070	-0.242	0.000		
Phosphorus	0.000059	0.001	0.008	0.909		
Sodium	-0.000051	0.000	-0.039	0.538		
Constant	3.671	0.248	-	0.000		

**Table G.9 (cont.)**

Variable		b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equation
<b>Model 3 : All factors</b>							
3.1	Age	0.233	0.042	0.362	0.000	0.389	0.53
	Sex	-0.137	0.073	-0.102	0.062		
	Weight/age	0.168	0.056	0.202	0.003		
	Height/age	0.134	0.058	0.156	0.023		
	Pre-puberty	-0.264	0.135	-0.177	0.051		
	Early-puberty	-0.381	0.096	-0.284	0.000		
	Usual calcium intake	0.00013	0.000	0.056	0.264		
	Weight bearing activity	0.0049	0.006	0.043	0.424		
	Constant	0.633	0.531	-	0.234		
3.2	Age	0.244	0.045	0.381	0.000		
	Sex	-0.172	0.071	-0.128	0.017		
	Weight/age	0.155	0.059	0.185	0.009		
	Height/age	0.157	0.059	0.181	0.008		
	Pre-puberty	-0.246	0.136	-0.165	0.071		
	Early-puberty	-0.388	0.097	-0.289	0.000		
	Current calcium intake	0.00018	0.000	0.033	0.511		
	Protein	-0.0246	0.063	-0.022	0.698		
	Phosphorus	-0.000549	0.000	-0.070	0.189		
	Sodium	0.000047	0.000	0.036	0.487		
	Constant	0.754	0.622	-	0.226		

**Table G.10** Multiple regression analysis of various factors on total body BMC

Variable	b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equation
<b>Model 1 : Physiological factors</b>						
Age	162.86	9.541	0.592	0.000	0.824	121.08
Sex	66.31	15.69	0.115	0.000		
Weight/age	144.45	12.71	0.402	0.000		
Height/age	62.01	13.19	0.167	0.000		
Pre-puberty	-114.49	30.73	-0.180	0.000		
Early-puberty	-128.33	22.01	-0.222	0.000		
Constant	-362.49	119.53	-	0.003		
<b>Model 2 : Environmental factors</b>						
2.1 Usual calcium intake	0.130	0.061	0.131	0.034	0.019	286.08
Weight bearing activity	3.57	3.05	0.072	0.242		
Constant	1264.46	44.93	-	0.000		
2.2 Current calcium intake	0.0116	0.076	0.009	0.879	0.004	287.93
Weight bearing activity	5.155	3.013	0.104	0.088		
Constant	1334.88	46.043	-	0.000		
2.3 Absolute value						
Current calcium intake	-0.0897	0.089	-0.072	0.313	0.011	286.85
Protein	1.735	1.813	0.120	0.339		
Phosphorus	0.0618	0.157	0.046	0.694		
Sodium	0.0095	0.023	0.031	0.676		
Constant	1236.34	65.673	-	0.000		
2.4 Nutrient density						
Current calcium intake	-0.169	0.133	-0.071	0.207	0.161	264.18
Protein	-196.716	27.447	-0.415	0.000		
Phosphorus	0.320	0.205	0.095	0.121		
Sodium	-0.048	0.033	-0.086	0.150		
Constant	1754.262	99.302	-	0.000		

**Table G.10 (cont.)**

	<b>Variable</b>	<b>b</b>	<b>SEb</b>	<b>BETA</b>	<b>p value</b>	<b>Adjusted R<sup>2</sup></b>	<b>SE of Equation</b>
<b>Model 3 : All factors</b>							
3.1	Age	160.20	9.74	0.582	0.000	0.825	120.97
	Sex	74.93	16.78	0.130	0.000		
	Weight/age	144.80	12.71	0.404	0.000		
	Height/age	58.75	13.32	0.158	0.000		
	Pre-puberty	-119.01	30.98	-0.187	0.000		
	Early-puberty	-129.41	22.10	-0.224	0.000		
	Usual calcium intake	0.0193	0.26	0.019	0.464		
	Weight bearing activity	2.149	1.409	0.043	0.128		
	Constant	-362.04	122.01	-	0.003		
3.2	Age	167.269	10.252	0.610	0.000	0.823	121.25
	Sex	65.406	16.361	0.114	0.000		
	Weight/age	143.483	13.429	0.399	0.000		
	Height/age	64.373	13.379	0.173	0.000		
	Pre-puberty	-108.901	31.214	-0.172	0.001		
	Early-puberty	-126.466	22.202	-0.220	0.000		
	Current calcium intake	0.110	0.063	0.046	0.084		
	Protein	-0.010	14.403	0.000	0.999		
	Phosphorus	-0.068	0.095	-0.020	0.474		
	Sodium	-0.0050	0.015	-0.009	0.747		
	Constant	-414.105	142.960	-	0.004		

**Table G.11** Multiple regression analysis of various factors on total body calcium

Variable	b	SEb	BETA	p value	Adjusted R <sup>2</sup>	SE of Equation
<b>Model 1 : Physiological factors</b>						
Age	61.872	3.624	0.592	0.000	0.824	45.99
Sex	25.166	5.959	0.115	0.000		
Weight/age	54.950	4.826	0.402	0.000		
Height/age	23.434	5.009	0.166	0.000		
Pre-puberty	-43.465	11.672	-0.180	0.000		
Early-puberty	-48.709	8.361	-0.222	0.000		
Constant	-137.60	45.399	-	0.000		
<b>Model 2 : Environmental factors</b>						
2.1 Usual calcium intake	0.049	0.023	0.130	0.034	0.019	108.66
Weight bearing activity	1.356	1.157	0.072	0.242		
Constant	480.588	17.065	-	0.000		
2.2 Current calcium intake	0.0044	0.029	0.009	0.878	0.004	109.36
Weight bearing activity	1.956	1.144	0.104	0.089		
Constant	507.228	17.488	-	0.000		
2.3 Absolute value						
Current calcium intake	-0.0340	0.034	-0.071	0.314	0.011	108.95
Protein	0.659	0.689	0.120	0.340		
Phosphorus	0.0234	0.060	0.046	0.694		
Sodium	0.00363	0.009	0.032	0.675		
Constant	469.81	24.943	-	0.000		
2.4 Nutrient density						
Current calcium intake	-0.0640	0.051	-0.071	0.208	0.161	100.34
Protein	-74.727	10.425	-0.416	0.000		
Phosphorus	0.121	0.078	0.095	0.121		
Sodium	-0.0182	0.013	-0.086	0.150		
Constant	666.555	37.715	-	0.000		

**Table G.11 (cont.)**

	<b>Variable</b>	<b>b</b>	<b>SEb</b>	<b>BETA</b>	<b>p value</b>	<b>Adjusted R<sup>2</sup></b>	<b>SE of Equation</b>
<b>Model 3: All factors</b>							
3.1	Age	60.87	3.70	0.582	0.000	0.825	45.95
	Sex	28.44	6.37	0.130	0.000		
	Weight/age	55.08	4.83	0.404	0.000		
	Height/age	22.21	5.06	0.158	0.000		
	Pre-puberty	-45.17	11.77	-0.187	0.000		
	Early-puberty	-49.12	8.40	-0.224	0.000		
	Usual calcium intake	0.0072	0.010	0.019	0.470		
	Weight bearing activity	0.814	0.535	0.043	0.130		
	Constant	-137.43	46.35	-	0.003		
3.2	Age	63.551	3.894	0.610	0.000	0.823	46.05
	Sex	24.827	6.214	0.114	0.000		
	Weight/age	54.586	5.100	0.400	0.000		
	Height/age	24.330	5.081	0.172	0.000		
	Pre-puberty	-41.333	11.855	-0.172	0.001		
	Early-puberty	-48.000	8.432	-0.219	0.000		
	Current calcium intake	-0.0419	0.024	0.046	0.083		
	Protein	-0.0037	5.470	0.000	0.999		
	Phosphorus	-0.0260	0.036	-0.020	0.474		
	Sodium	-0.0019	0.000	-0.009	0.746		
	Constant	-157.254	54.296	-	0.004		

**BIOGRAPHY**

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