University of Mosul

College of Medicine

Changes in Bone Mineral Density During Puberty

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Summary

Bone mineral density (BMD) increases during growth to reach a peak in young adulthood, plateaus, and declines thereafter. Growing awareness that osteoporosis may have its antecedents in childhood and adolescence has led to increasing interest in assessing BMD during puberty. Several noninvasive imaging techniques are currently available to measure BMD, the most widely used technique is dual-energy x-ray absorptiometry (DXA).

The present study aimed to determine the changes of BMD, growth parameters [weight, height and body mass index (BMI)], serum total calcium, serum inorganic phosphorus, serum total alkaline phosphatase (ALP) actvity and serum sex steroids[testosterone for males and estradiol (E_2) for females] in healthy pubertal males and females from Mosul city/Iraq at different pubertal stages. The study also aimed to detect the relationship of BMD with the other parameters, and to reveal the most important determinant of BMD in the pubertal period. Furthermore, it aimed to allow an insight into gender dimorphism in mineral accretion during puberty.

This study was conducted in Ibn-Sina teaching hospital in Mosul from November 2010 to March 2011. BMD of the lumbar spine was performed using DXA to 177 healthy pubertal children and adolescents, including (96) males and (81) females aged (10-20.2) years.

The study population were generally healthy, without a history of disease or therapy which could interfere with bone metabolism. The participants and their parents were all informed about the study protocol and seek consent. Height and weight were measured, BMI was calculated. Pubertal stages were assessed and the subjects were classified into five pubertal stages from I to V. Serum calcium, phosphorus and ALP were

measured colorimetrically. Sex steroids (testosterone for males and E_2) for females) were measured by Minividas, using Enzyme Linked Fluorescent Assay technique (ELFA).

This study provided gender-specific lumber spine BMD values, expressed in discrete age and pubertal stage subgroups. A progressive increase in BMD values in the different age groups. Rapid accumulation of BMD in the lumbar spine was observed around age 13 and 15 years in females and males, respectively. Lumbar BMD was higher in females in all age groups, probably because of the earlier onset of puberty in females.

A significant increase in BMD values according to puberty was observed among all pubertal stages concomitant with the significant increase in body dimensions and its relationship with maturation of secondary sexual characteristics. Both genders showed the main increase in BMD between stage IV and V. There were no significant differences between them except in stage III, in that males had higher BMD values than females, this indicated that males gain more BMD than females at this stage due to significant increase in testosterone level at this stage.

The major determinants of BMD in both genders were pubertal stage and sex steroids, which significantly increased during puberty. Furthermore, growth parameters were predictors for BMD values in males only but not in females.

Serum phosphorus and ALP levels varied significantly with pubertal stages being higher at early puberty (stage I and II) and declined in mid and late puberty (stage III, IV and V). In contrast to calcium which remained within the same range in all stages. Serum phosphorus, calcium and ALP levels were not predictors for BMD in both genders.

BMD values for the study group were lower than Western normative values, with Z- scores: -1.2±1.2 for females and for -1.4±1.1 for males.

In conclusion, BMD increased throughout puberty, and pubertal stage was the major determinant of BMD in both genders. Growth parameters were good predictors of BMD in males, but not in females. Testosterone levels in males and E_2 levels in females made the greatest contribution to BMD acquisition.

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List of Abbreviations

Abs	Absorbance
ALP	Alkaline phosphatase
AR	Androgen receptor
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BMU	Basic multicellular units
CaR	Calcium-sensing receptor
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
DXA	Dual-energy x-ray absorptiometry
E_1	estrone
E_2	17β estradiol
E_3	estriol
ECF	Extracellular fluid
ELFA	Enzyme Linked Fluorescent Assay technique
ER	Estrogen receptor
GM-CSF	Granulocyte/Macrophage-colony stimulating factor
GH	Growth hormone
3 β-HSD	3 β-hydroxysteroid dehydrogenase
17 β-HSD	17 β-hydroxysteroid dehydrogenase
IGF-I	Insulin-like growth factor
M-CSF	Macrophage-colony stimulating factor
NS	Non significant
OPG	Osteoprotegerin
PBM	peak bone mass
PBV	Peak bone velocity
PGE-2	Prostaglandins-E-2
PHV	Peak height velocity
PTH	Parathyroid hormone
PTHR	Parathyroid hormone receptor
R	Reagent
RANK	Receptor activator of nuclear factor-kappa beta
RANKL	Receptor activator of nuclear factor-kappa beta-ligand
SD	Standard deviation
SHBG	Sex hormone-binding globulin

SPR	solid phase receptacle
Sv	Sievert
TGF-β	Transforming growth factor beta
TNF	Tumor necrosis factor
VDR	Vitamin D receptor

Bone physiology

Bone is a dynamic multifunctional organ that is comprised of a structural framework of calcified matrix containing populations of many cell types including chondrocytes, osteoblasts, osteocytes, osteoclasts, endothelial cells, monocytes, macrophages, lymphocytes, and hematopoeitic cells. Bone is constantly regenerated throughout life as a consequence of bone turnover in response to hormonal signals, paracrine and autocrine factors, and physical stresses (Grzibovskis *et al.,*2010).

Types of the bone: Bone in children and adult is of two types:

1- **Cortical or compact bone** makes up the outer layer of most bones providing a strong protective layer and account for 80% of total bone mass (Boron and Boulpaep,2005). Cortical bone is made up of functional units called osteons, which contain layers of bone matrix, or lamellae, that surround central canals called Haversion canals. These canals contain blood vessels and generally run parallel to the surface of the bone (Steven and Lowe,1997).

2- **Trabecular or cancellous bone** is found in the interior of bones; it is especially prominent within the vertebral bodies. It represents the remaining 20% of the total bone mass (Barrett *et al*.,2010). The composition of the bone matrix is the same for cortical and cancellous bone. However, in cancellous bone there are no osteons, and the matrix forms interconnecting plates called trabeculae which make the surface area of cancellous bone approximately twenty times more than cortical bone, and thus has a higher rate of bone remodeling. About 5% of cortical bone and 20% of cancellous bone is renewed per year. Therefore, cancellous bone is considered more susceptible to changes in mechanical loading and bone loss with aging (Steven and Lowe,1997).

Bone matrix:

Bone is composed of cells lying in an extracellular matrix that has become calcified. Bone matrix has inorganic and organic constituents:

» **Inorganic component** is mainly composed of calcium and phosphorus which exist in the form of hydroxyapatite crystals [$Ca_{10}(PO_4)_6(OH)_2$]. These crystals are arranged in an ordered fashion along the collagen fibers, and this association of hydroxyapatite crystals with collagen gives the bone its hardness and strength (Boron and Boulpaep,2005).

» **Organic component** consists of 90% collagen and about 10% of various non-collagenous proteins such as osteocalcin, osteonectin, osteopontin and bone sialoprotein. The urinary excretion and serum levels of some of the non-collagenous proteins unique to bone are used clinically to assess bone turnover. The collagen fibers provide flexibility that allows bone to respond to forces by bending rather than breaking (Gartner and Hiatt,2001).

Bone cells:

The structure of bone is formed, maintained and reformed by the collective action of cells that produce and mineralize bone matrix, and degrade it. These cells are:

• **Osteoblasts** are derived from multipotent mesenchymal stem cells in the bone marrow. They are responsible for the formation of structural components of bone (i.e., matrix and mineral) and the production of regulatory factors that influence bone formation and resorption.

• **Osteocytes** are mature bone cells, derived from osteoblasts that are housed in lacunae within the calcified bone matrix. Although they appear to be inactive cells, they may be responsible for control the movement of

ions in and out of the matrix and the detection of stresses on bone. These cells respond to stimuli that place tension on bone by releasing of growth factors as insulin-like growth factor (IGF). The release of these factors facilitates the recruitment of proosteoblasts (precursors of osteoblast) to assist in the remodeling of the skeleton.

• **Osteoclasts** are multinucleated cells originating in the bone marrow from hematopoietic stem cells of monocyte-macrophage lineage. Osteoclasts have receptors for interleukins (IL), granulocyte/macrophage-colony stimulating factor (GM-CSF), macrophage-colony stimulating factor (M-CSF) and calcitonin. These cells are responsible for resorbing bone by producing hydrogen ions to mobilize minerals and lysosomal enzymes to digest the organic matrix. After they finish doing so, they undergo apoptosis (Gartner and Hiatt,2001).

Bone Turnover

Bone is constantly being turned over. There are two processes of bone turnover; modeling, and remodeling:

Bone modeling:

It is the process by which bones grow in size and shape, and is responsible for maintaining the characteristic morphology of the growing bone during puberty. It occurs during the first two decades of life while growth plates remain open (Henwood and Binkovitz,2009). Bone formation during this period mostly results from modeling, although bone remodeling also occurs. In the mature adult skeleton, modeling may occur in response to altered biomechanical stress such as that induced by vigorous exercise and also occurs as part of the fracture healing process (Tuchman *et al*.,2008). The process of bone modeling involves both bone

formation and resorption; the former exceeds the latter and is not coupled to it temporally or spatially as in bone remodeling (Compston, 2001). Modeling generally modifies the shape of the bone by periosteal or endosteal apposition. Periosteal apposition defines the cross sectional area of bone through the action of osteoblasts that add mineralized tissue on the outer bone surface, while endosteal apposition determines cortical thickness (Rauch,2007; Henwood and Binkovitz,2009).

Bone remodeling:

It is a continues process that enable bone to maintain normal skeletal mass of the adult, repair microdamage to the skeleton and participate in calcium and phosphorus homeostasis (Grzibovskis *et al*.,2010).

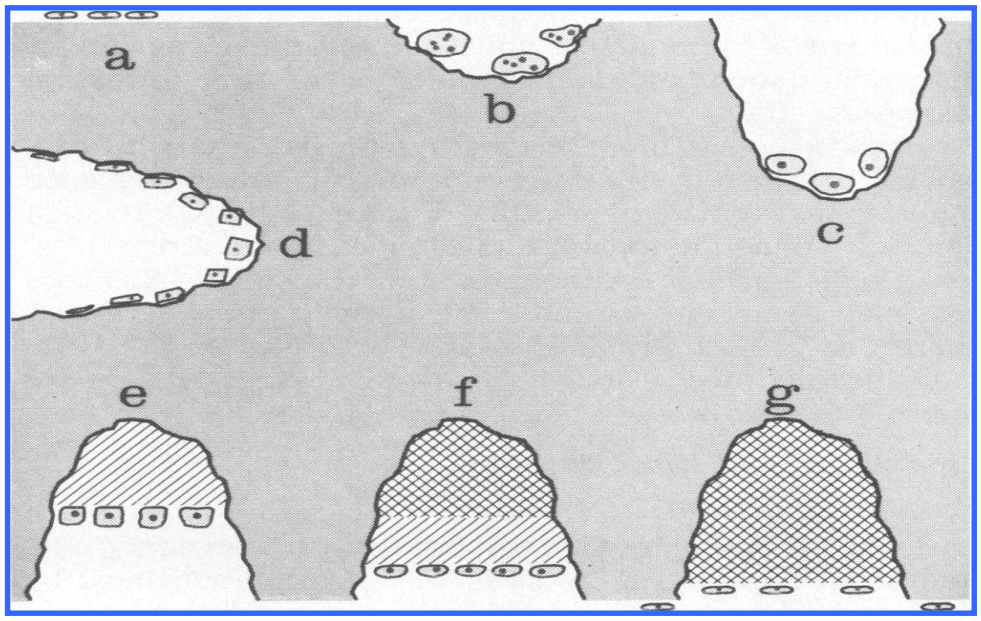


Figure (1): The bone remodeling cycle (Marcus,1991).

a- resting trabecular surface;
b- multinucleated osteoclasts dig a cavity;
c- resorption is completed by mononuclear phagocytes;
d- osteoblast precursors are recruited to the base of the resorption cavity;
e- new matrix is secreted by osteoblasts;
f- matrix continues to be secreted, with the initiation of calcification;
g- mineralization of the new matrix is completed. Bone has returned to a quiescent state.

Remodeling cycle comprises five phases: resting, activation, resorption, reversal and formation (figure-1), this carried out by coordinated groups of osteoblasts and osteoclasts, called basic multicellular units (BMU), that is, bone formation by osteoblasts and bone resorption by osteoclasts are tightly coupled in time and space within the BMU (Hernández-Gil *et al*.2006), this cycle takes about 100 days (Barrett *et al*,2010).

Regulation of bone modeling and remodeling

Bone modeling and remodeling are regulated by systemic hormones and local factors which affect cells of the osteoclast or osteoblast lineage.

Systemic hormones - include the following hormones:

*** Parathyroid hormone (PTH)**

*** vitamin D**

*** sex steroids**

*** calcitonin**

PTH, vitamin D, calcitonin and sex steroids will be discussed later on.

* **Insulin**: It causes stimulation of bone matrix synthesis and cartilage formation, and it is also necessary for normal bone mineralization.

* **Growth hormone** (GH) : It has two main actions on bone: one direct, through the stimulation of chondrocytes proliferation on the growth plate, and one indirect, through the stimulation of osteoblast formation and activity via the production of IGF-I and IGF-II. In several studies, GH has been shown to elevate circulating levels of osteocalcin (a marker of bone formation). Thus, GH or its surrogate, IGF-I, might also be useful to increase bone mineral density (BMD). Moreover, children who are GH deficient, have been found to have reduced bone size and a lower BMD than healthy age matched peers (Grzibovskis *et al*.,2010).

* **Glucocorticoids**: Recent studies have demonstrated that at physiological doses, glucocorticoids have an osteogenic capacity favoring osteoblastic differentiation. While, at pharmacologic doses, they promote bone resorption by inhibiting the synthesis of IGF-I by the osteoblasts, accelerating the maturation and activity of osteoclasts and exerting antiapoptotic effects on these cells. In addition, glucocorticoids reduce intestinal calcium absorption and promote renal calcium excretion (Hernández-Gil *et al*.,2006).

* **Thyroid hormone**: it has two opposing actions on bone. It stimulates the synthesis of the osteoid matrix by the osteoblasts and its mineralization, favoring the synthesis of IGF-I. A contrary effect, in that it stimulates resorption by increasing number and function of the osteoclasts (Hernández-Gil *et al*.,2006).

* **Leptin**: it is produced in adipose tissue, it stimulates the differentiation and proliferation of osteoblasts and suppresses osteoclastogenesis by activating monocyte production of IL-1 receptor antagonists (IL-1 is a stimulator of bone resorption) and by increasing the production of osteoprotegerin (OPG) which is an inhibitor of bone resorption (Grzibovskis *et al*.,2010).

Local factors:

Numerous cytokines and growth factors produced by the bone cells themselves or in extra-osseous tissue, act as modulators of the cellular functions, fundamentally growth, differentiation, and proliferation, these factors include:

* **IGF-I and II**: these are polypeptides similar to insulin; they are synthesized by the liver and osteoblasts, and found in high concentrations in the osteoid matrix. They have osteogenic effect by enhancing bone

collagen and matrix synthesis and stimulate the replication of cells of osteoblast lineage. IGF synthesis is regulated by hormones and local growth factors; GH and estrogens, for example, increase its production, while the glucocorticoids inhibit it (Eastell, 2005).

* **IL-1**: It is released from osteoblasts, it stimulates bone resorption by increasing the osteoclastic activity as well as inhibiting the apoptosis of the osteoclasts.

* **IL-6**: It is released from osteoclasts, it is believed to play an important role in the initial stages of osteoclastogenesis.

* **IL-11**: it is produced in bone marrow and induces osteoclastogenesis (Hernández-Gil *et al*.,2006).

* **Interferon-γ**: It is released by T-lymphocytes, inhibits differentiation of osteoclast precursors into osteoclasts (Eastell, 2005).

* **Transforming growth factor beta (TGF-β)**: It is librated from bone matrix and is regarded as a potent stimulator of bone formation by promoting osteoblastic proliferation and the synthesis of the osteoid matrix. Also, TGF-β inhibits osteoclast activation and bone resorption (Grzibovskis *et al*.,2010).

* **Fibroblastic Growth Factor**: It has an anabolic effect on bone, it is released from osteoblasts, vascular endothelial cells, and fibroblasts.

* **GM-CSF**: It is important in osteoclastogenesis.

* **M-CSF**: It is produced by osteoblasts and their precursors. It is an essential factor in the first phases of osteoclastogenesis, but has no effect on osteoclastic activity (Hernández-Gil *et al*.,2006) .

* **Tumor necrosis factor (TNF) family**: Recent molecular approaches have rapidly advanced the field by the discovery and extensive characterization of three new cytokine systems of the (TNF) family. They have been found to regulate proliferation, differentiation, activation, and

apoptosis of osteoclasts. This system is comprised of a ligand; RANKL (receptor activator of nuclear factor - kappa beta ligand) and also its specific receptor; RANK (receptor activator of nuclear factor-kappa beta), as well as decoy receptors; OPG. RANK is a protein receptor on the membrane surface of macrophages (Grzibovskis *et al*.,2010).

Osteoblasts and their precursors produce two chemical signals (RANKL and OPG) that govern osteoclast development and activity. Binding of RANKL to RANK induces the macrophages to differentiate into osteoclasts and suppresses their apoptosis, while binding of OPG to RANKL, prevents RANKL from binding with its intended RANK receptors and suppresses osteoclast development and activity (figure-2).

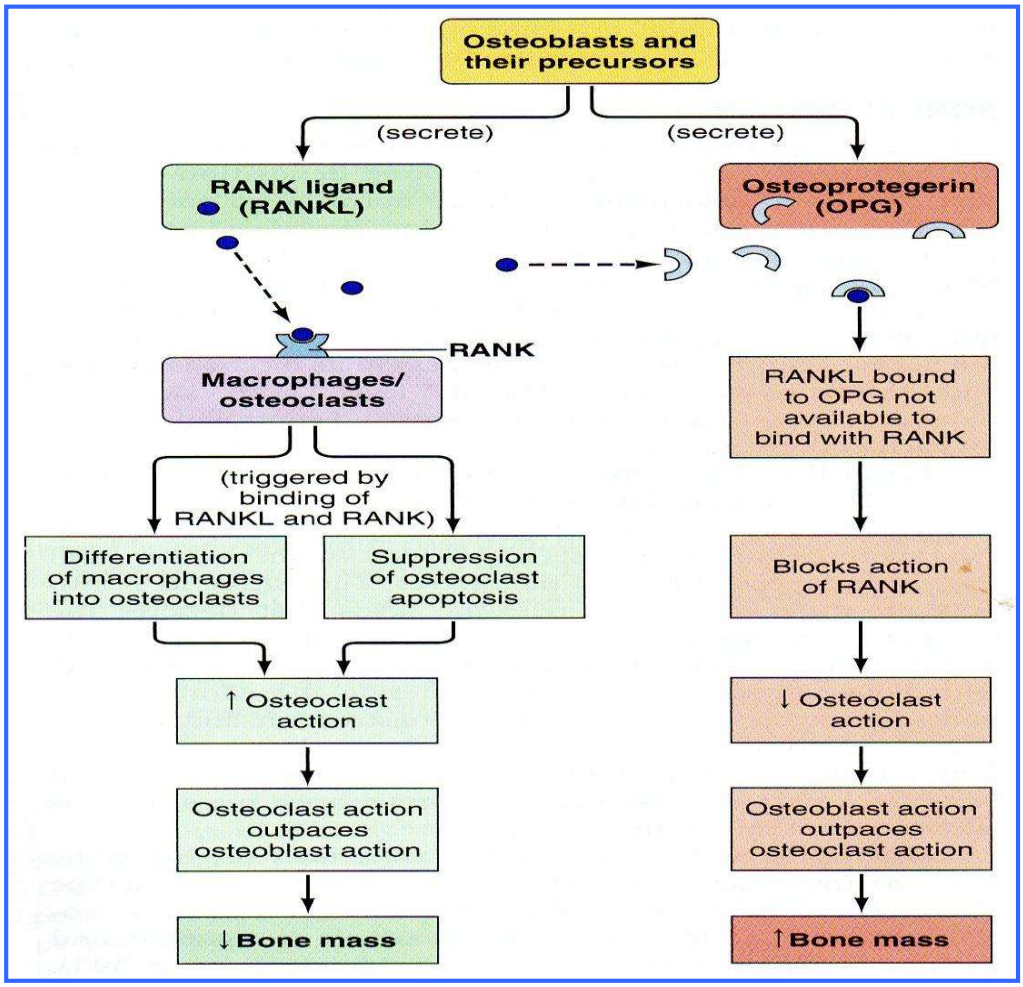


Figure (2): Role of osteoblasts in governing osteoclast development and activity (Sherwood,2007).

The balance between RANK and OPG is thus an important determinant of BMD. If osteoblasts produce more RANKL, the osteoclast action is increased and the BMD is reduced. If osteoblasts produce more OPG, the osteoclast action is reduced and the BMD is increased. There are many factors affecting this balance. Estrogen, for example, stimulates activity of the OPG- producing gene in osteoblasts, which is one of mechanisms by which this hormone preserves bone mass, whereas glucocorticoids increase the production of RANKL by osteoblasts but decrease the production of OPG, so they promote bone loss (Boron and Boulpaep,2005; Sherwood,2007; Grzibovskis *et al*.,2010).

Calcium

Calcium is the fifth most abundant element in the human body, it is important for the function of heart, nervous system, muscle contraction and coagulation process. Ninety nine % of the total calcium is in the skeleton. Of the remaining 1%, about 0.9% is found intracellularly within the soft tissues, less than 0.1% is present in the extracellular fluid (ECF).

Plasma calcium, normally at a concentration of around 10 mg/dl (2.5 mmol/L), is present in three forms in the plasma:

1- About 41% (1 mmol/l) is combined with plasma protein; it is non diffusible through the capillary membrane.

2- About 9% (0.2 mmol/l) is diffusible through capillary membrane but is combined with anionic substances (citrate and phosphate, for instance) in such a manner that it is not ionized.

3- The remaining 50% (1.2 mmol/l) of the calcium in plasma is both diffusible through the capillary membrane and ionized; so only this free calcium is biologically active and subject to regulation (Sherwood,2007; Barrett *et al*.,2010).

In bone, calcium serves two main purposes: it provides skeletal strength and, concurrently, provides a dynamic store to maintain the intra- and extracellular calcium pools. The calcium in bone is of two types: a readily exchangeable reservoir and a much larger pool of stable calcium. The exchangeable calcium is about 0.4-1% of total bone calcium and it is deposited in bones in a form of readily mobilizable salt such as $CaHPO_4$. The importance of exchangeable calcium is that it provides a rapid buffering mechanism to keep the calcium ion concentration in the ECF within normal ranges (Guyton and Hall,2006; Barrett *et al*.,2010)

Control of calcium metabolism

It depends on hormonal control of exchanges between the ECF and three other compartments: bone, kidneys and intestine. Control of calcium metabolism encompasses two aspects**:**

Calcium balance: It involves the more slowly responding adjustments required to maintain a constant total amount of calcium in the body. Control of calcium balance ensures that calcium intake is equivalent to calcium excretion over the long term (weeks-months) (Sherwood,2007).

Calcium balance is a function of dietary intake, intestinal absorption, renal excretion, and bone remodeling. Bone balance changes throughout the normal lifespan, depending on a number of factors, including growth, aging, and acquired or inherited disorders. Children are in positive bone balance (formation > resorption). Healthy young adults are in neutral bone balance (formation = resorption) and have achieved peak bone mass (PBM). Elderly individuals are typically in negative bone balance (formation < resorption), which leads to age- related bone loss (Rauch,2007; Peacock,2010).

Regulation of serum calcium homeostasis: It refers to the hormonal regulation of serum ionized calcium on a minute-to-minute basis. It involves three hormones and their receptors: PTH and the PTH receptor (PTHR), 1,25-dihydroxyvitamin D and the vitamin D receptor (VDR), the calcium-sensing receptor (CaR) and calcitonin.

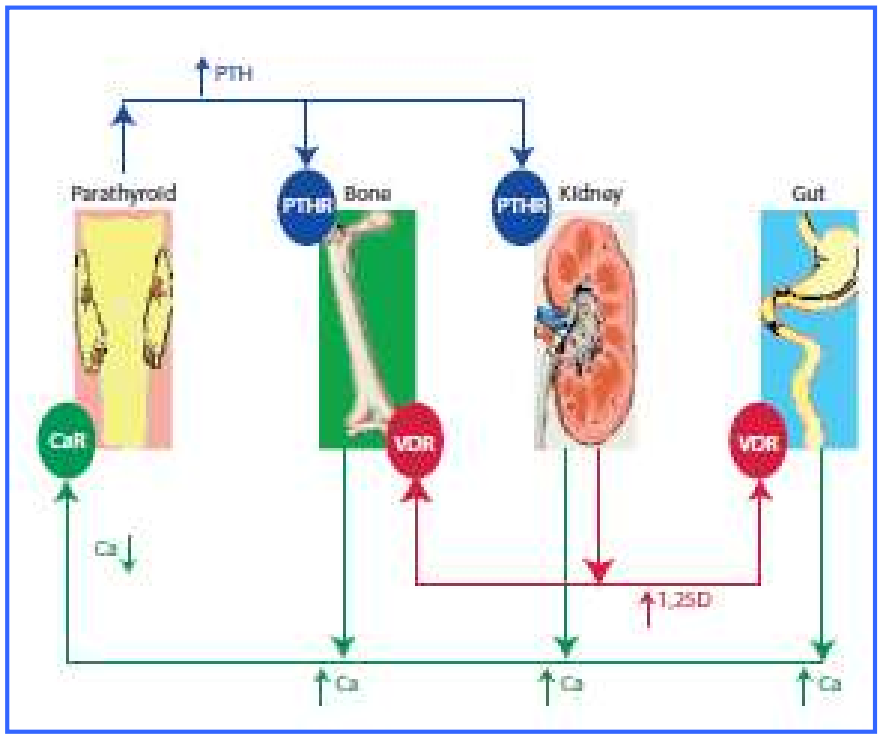


Figure (3): Regulation of serum calcium homeostasis (Peacock,2010).

In figure (3), a decrease in serum calcium inactivates the CaR (present in the parathyearoid cells) to increase PTH secretion, which acts on the PTHR in kidney to increase tubular calcium reabsorption, and on the PTHR in bone to increase net bone resorption. The increased PTH also stimulates the kidney to increase secretion of vitamin D, which activates the VDR in gut to increase calcium absorption, calcium is absorbed in the small intestine by both passive and active processes, active process requires vitamin D (Sherwood,2007; Peacock,2010).

Parathyroid hormone (PTH)

PTH is a peptide hormone, secreted from chief cells in parathyearoid gland. It provides a powerful mechanism for controlling extracellular calcium and phosphorus concentrations by regulating intestinal reabsorption, renal excretion, and exchange between ECF and bone of these ions. The following are the main actions of PTH:

In the kidney: PTH promotes the reabsorption of calcium in the thick ascending limb and distal convoluted tubules, promotes phosphorus loss and also stimulates the 1-hydroxylation of 25-hydroxyvitamin D in the proximal tubule, the resulting 1,25-dihydroxycholecalciferol is the most biologically active vitamin D, having a synergistic action of promoting calcium reabsorption in the distal convoluted tubules (Boron and Boulpaep,2005; Guyton and Hall, 2006) .

In the bone: PTH promotes both bone resorption and bone formation, however, the net effect of PTH on bone is to stimulate bone resorption, thus increasing plasma calcium. In bone the osteoblast appears to be the important target cell, expressing surface receptors for PTH, but osteoclasts do not. PTH stimulates osteoblast to release agents such as M-CSF and stimulate the expression of RANKL, which promote the development of osteoclasts. Moreover, PTH and vitamin D stimulate osteoblasts to release IL-6, which stimulates existing osteoclasts to resorb bone. On the other hand, PTH promotes bone formation, although to lesser extent, either directly or indirectly. Direct effect of PTH by activating calcium channels in osteocytes, leading to transfer of calcium from bone fluid to the osteocytes, and then to the osteoblasts (via gap junctions), which pump this calcium into the extracellular matrix, thus contributing to mineralization. PTH stimulates bone synthesis indirectly in that bone resorption leads to release of growth factors such as IGF-I, IGF-II, and TGF-β (Boron and Boulpaep,2005; Sherwood,2007).

Vitamin D: The actions of vitamin D on the intestine, bone and kidney serve to prevent any abnormal decline or rise in serum calcium. It has been recognized for long time that vitamin D is important for bone mineralization, though there is little direct evidence that vitamin D actively participates in this process (because the direct action on bone is to mobilize calcium out of the bone); Instead, vitamin D promotes the mineralization indirectly by promoting calcium and phosphorus reabsorption in the distal convoluted tubules and increasing their absorption by the small intestine. So it raises the concentration of calcium and phosphorus in the blood and ECF, and hence results in deposition of calcium hydroxyapatite into the bone matrix. These indirect effects overshadow the direct effect of vitamin D to increase bone mobilization (Favus,1996; Boron and Boulpaep,2005).

Calcitonin: It is the third hormone that control calcium metabolism, it is a peptide hormone secreted by the thyroid gland. It tends to decrease plasma calcium concentration by inhibiting osteoclastic bone resorption (Guyton and Hall, 2006).

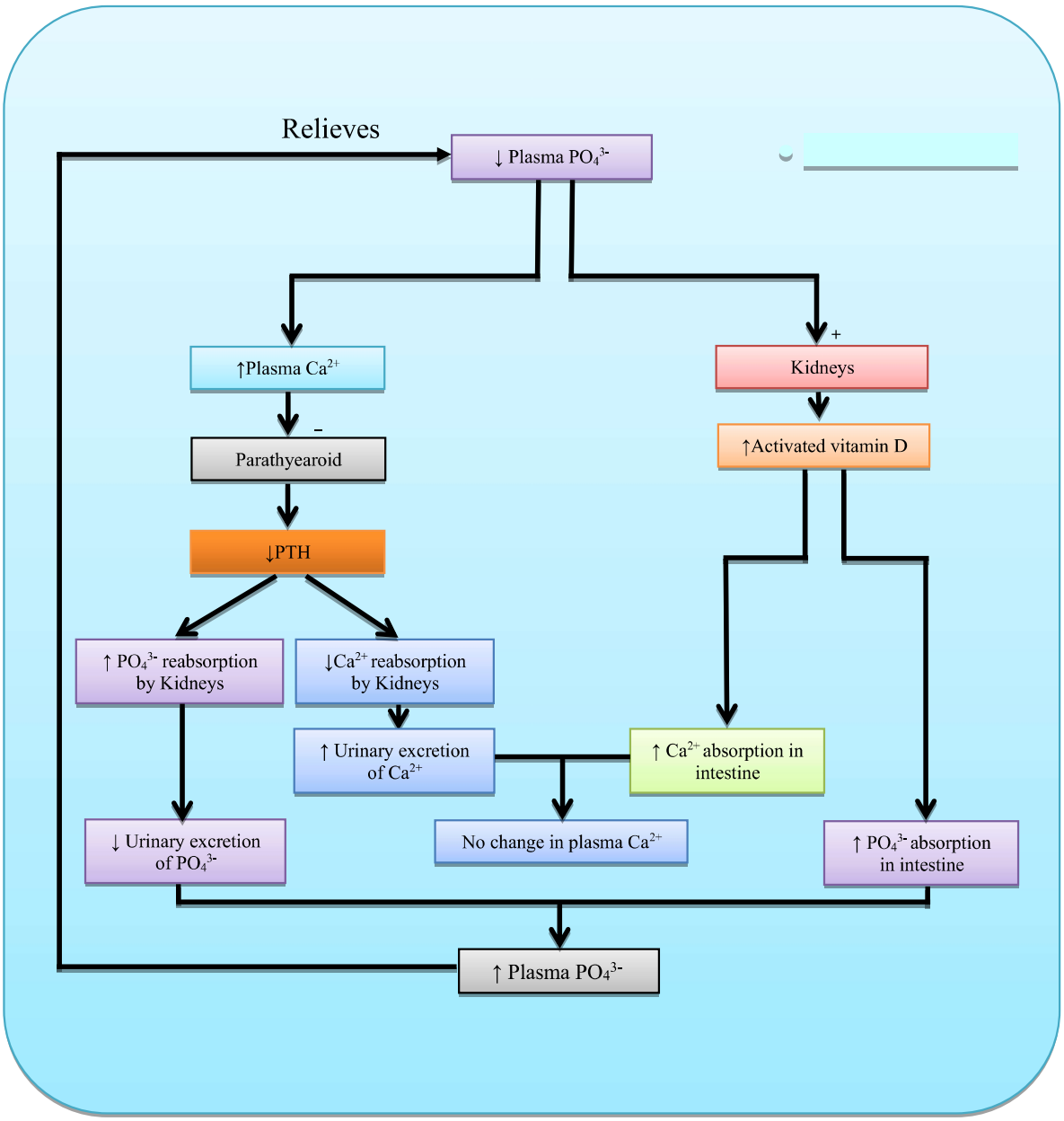
Phosphorus

Phosphorus is a major component of bone and other tissues. The skeleton contains 85-90% of total body phosphorus, 14-15% is in the cells, and less than 1% is in the ECF. Phosphorus exists in plasma in two forms: an organic form (principally consisting of phospholipids) and an inorganic form. About 15% of inorganic form is protein bound, the remaining 85% exists as free ions, HPO_4^- and $H_2PO_4^-$. In clinical settings, only the inorganic form is routinely measured (Barrett *et al.*,2010).

Serum inorganic phosphorus level is age-depending, and children have much higher values than adults. The average serum phosphorus is about 1.20-1.75 mmol/l in children aged 7-15 years, and 0.90-1.50 mmol/l in adolescents aged 16-19 years (Mclntosh,2008).

Control of Phosphorus metabolism:

Phosphorus Balance: It includes both the organic and inorganic forms. Phosphorus balance, like calcium, is also maintained by intestinal absorption, renal excretion, and bone accretion (Favus,1996).



Figure(4):Regulation of serum phosphorus homeostasis (Sherwood,2007).

Regulation of serum Phosphorus homeostasis:

Phosphorus is regulated directly by vitamin D and indirectly by the plasma calcium-PTH feedback loop. A fall in plasma phosphorus increases activation of vitamin D, which promotes phosphorus absorption in the intestine, returning plasma phosphorus toward normal. Moreover, because of the inverse relationship between phosphorus and calcium concentrations in the plasma caused by solubility characteristics of calcium phosphate salts, a fall in plasma phosphorus increases plasma calcium, which suppresses PTH secretion and then increases in phosphorus reabsorption by the kidneys (figure-4).

Note that these changes do not compromise calcium balance, in that the increase in activated vitamin D stimulates calcium absorption and the concurrent fall in PTH produces a compensatory increase in urinary calcium excretion because less of the filtered calcium is reabsorbed (Sherwood,2007; Peacock,2010).

Alkaline phosphatase (ALP)

Alkaline phosphatases (ALPs) are a group of enzymes, found in many tissues, including bone, liver, placenta, kidney, intestine. They are enzymes of hydrolase class that catalyze the cleavage of phosphate groups from the macromolecules of the matrix under alkaline conditions, increasing the concentration of this ion in the microenvironment. The ALPs from liver and bone are isoforms of the same gene product, they have identical amino acid sequences, so it is much more difficult to distinguish between them (Burtis *et al*.,2008).

Bone ALP plays a key role in bone mineralization, it generates phosphate ions from organic phosphates but the precise function of ALP is not absolutely defined. It is a prominent product of osteoblasts and their precursors in different stages of differentiation. Bone ALP

concentration in the serum is used as an index of bone formation, elevated levels in the blood indicate excessive osteoblast activity.

Bone ALP exhibits a marked age-dependency, in growing children it constitutes about 75-90% of total ALP activity in the serum, after puberty this proportion declines to 50% (Rogol,2002; Schoenau and Rauch,2003). The measurement of ALP has several advantages over other markers because its relatively long half-life in vivo (1-3 days), it is unaffected by diurnal variation, it is more stable in vitro and does not require special specimen handling, also it is more useful in patients with impaired renal function because it is cleared mainly via liver (Burtis *et al*.,2008).

Puberty

Puberty in human is a unique and integrated transition from childhood to young adulthood. It involves major physical, emotional and psychological changes that culminate in the attainment of fertility (Bond *et al*.,2006). Puberty is characterized by development of the secondary sexual characters for each gender as well as major alterations in linear growth, body composition and the regional distribution of body fat, so it is the time of greatest growth and sexual development since the fetal stages (Rogol,2002; Mejia *et al*.,2009).

Childhood is defined as the period between infancy and the onset of puberty. On the other hand, adolescence refers to the time after pubertal onset during which a mature reproductive system is attained (MacKelvie *et al*.,2002).

Puberty occurs due to the increase in the secretion of the hypothalamic factor (gonadotrophin-releasing hormone). This in turn acts on the pituitary through gonadotrophin-releasing hormone receptors, thus stimulates the pulsatile secretion of gonadotrophin, luteinizing hormone

(LH) and follicle-stimulating hormone (FSH). Both of them act on gonads to stimulate the secretion of testosterone with the development of spermatogenesis in boys, and to stimulate the secretion of estrogen with development of ovarian follicle in girls (Sizonenko,2002).

The onset of puberty is around the age of 11 years in girls and 13 years in boys. Girls start and complete each stage of puberty earlier than boys do (Rogol *et al*.,2000). The factors responsible for initiating puberty remain unclear, although multiple intrinsic and extrinsic factors play a role, genetic factors are major determinants of pubertal onset, other factors such as nutrition, geographic location, exposure to light and body composition `(Pantsiotou,2007). Recent studies focus on a potential role for the hormone melatonin, in the initiating the onset of puberty in humans. Melatonin, which is secreted by the pineal gland, has an antigonadotropic effect in many species (Sherwood,2007).

Stages of maturity in childhood and adolescence

There are many methods that are commonly used to assess child´s maturity, such as:

- Skeletal age by standard radiography, which is rarely used because it involves the use of ionizing radiation.
- The size of uterus by ultrasonographic examination is another way for assessing pubertal development in females; but it is not so precise method (Shirtcliff *et al*,2009).
- Tanner staging by self assessment is non-invasive and has approximately the same degree of precision as radiograph (MacKelvie *et al*.,2002).

In 1962 Tanner described five stages of puberty, ranging from I (no development) to V (adult development). These stages capture visible

secondary sexual characteristics such as breast, genital development and pubic hair growth. Tanner's sexual maturation scale (SMS) is used to be the gold standard for assessing pubertal status (Bond *et al*.,2006; Shirtcliff *et al*.,2009).

Children in Tanner stage I are considered prepubertal, Tanner stages II and III represent the early pubertal phases, in Tanner stage IV adolescents are considered to be in the late stages of puberty, whereas Tanner stage V represents full maturity, or the postpubertal adolescents. There is a wide range of chronological ages within each Tanner stage (MacKelvie *et al*.,2002).

In a normal male, the first sign of puberty (stage II) is the enlargement of the testes to more than 2.5cm. Testosterone is an excellent parameter for the determination of the onset of puberty, most of the boys who have started puberty have testosterone levels above 0.25 ng/ml. In a normal female, the first sign of puberty (stage II) is the appearance of breast bud. Breast development is the most sensitive biological sign for assessing the secretion of estrogen. In most girls, menarche is achieved after Tanner stage III, and most commonly observed in girls at age 12.7 years (Sizonenko,2002; Boron and Boulpaep,2005).

Pubertal growth spurt

Pubertal growth spurt means a sudden acceleration of growth during midpuberty, it is a time of significant height, weight and BMD gain, that is, peak height velocity (PHV), peak weight velocity and peak bone velocity (PBV). It is one of the hallmarks of puberty occurred at Tanner stage III in girls and at stage IV in boys. PHV averages 10.3 cm/year in boys and 9 cm/year in girls, while peak weight velocity averages 9 kg/year in boys and 8.3 kg/year in girls (Rogol *et al*.,2000; Karen,2000).

After this spurt, there is a period of decelerating height, weight and BMD velocity, and then, growth virtually ceases because of epiphyseal fusion, at age of 15 years in girls and 17 years in boys, although small increases in BMD continue until the PBM is achieved (Riggs *et al*.,2002).

Compelling evidence indicates that the sex hormones enhance the axis of GH and IGF-1 in both sexes which is the main mediator of the accelerated growth, thus, sex steroids initiate the pubertal growth spurt and then end it by inducing epiphyseal closure. GH and IGF-I are maintained at high pubertal levels during the 3–4 years of rapid growth but then gradually decrease to prepubertal levels over several years, although the serum sex steroids remain at adult levels (Jarvinen *et al*.,2003; Pantsiotou,2007).

Sex steroids

Sex steroids are mainly synthesized by the gonads (testis and ovary) but the adrenals constitute an additional source, the majority of them circulate bound to albumin or to sex hormone-binding globulin (SHBG), a specific carrier protein synthesized by the liver (Nilsson *et al*.,2001).

Sex steroids are secreted during childhood, but the levels are too low to stimulate bone growth and the development of secondary sex characteristics. During early puberty (Tanner stage II to III), sex steroids levels begin to rise, and both are produced by each gender, however androgen levels are higher in boys and estrogen levels are higher in girls (Riggs *et al*.,2002).

Although the primary function of the sex steroids is reproduction, they have an important impact on bone physiology and on the development and maintenance of skeleton. The main actions of sex steroids on bone include: control of growth plate maturation and closure

during longitudinal bone growth, sexual dimorphism of the skeleton, regulation of cortical and cancellous bone metabolism, acquisition of PBM and maintenance of BMD in adults. Although the mechanisms of these actions are still not entirely clear; their effects on the acquisition of BMD can be direct, as well as indirect through modulation of GH, IGF-I and numerous cytokines and local growth factors (Hofbauer and Khosla,1999; Bertelloni *et al*.,2010)

People with disorders of sex development may experience some problems in developing normal bone growth, structure and mass. Adolescents with delayed puberty or females with secondary amenorrhea may fail to accrue BMD normally. Moreover, there are extensive data indicating that severe hypogonadism is associated with a low BMD resulting from inadequate BMD accrual during puberty, this low BMD is rapidly increased by sex steroids replacement therapy (Abu *et al*.,1997; Doneray and Orbak,2008; Bertelloni *et al*.,2010).

Estrogens

The most important endogenous estrogens are estrone (E_1), 17β estradiol (E_2), and estriol (E_3), E_2 is the most potent and important estrogen in female. The cyclical secretion of estrogen normally begins in early adolescence and continues throughout the entire fertile period (excluding periods of pregnancy) until the eventual cessation of female reproductive capability.

Small increases in estrogens are probably the earliest marker of pubertal changes before physical changes are observed, marked increase in estrogens is between 10-12 years of age and plasma concentration above 25 pg/ml suggests pubertal ovarian secretion. The onset of estrogen secretion at puberty induces extrapacking of bone mineral into female

skeleton which is regarded as a safety measure against the anticipated bone loss caused by pregnancy and lactation (Abrams *et al*.,2000; Sizonenko,2002; Jarvinen *et al.*,2003).

Estrogen receptors (ERs)

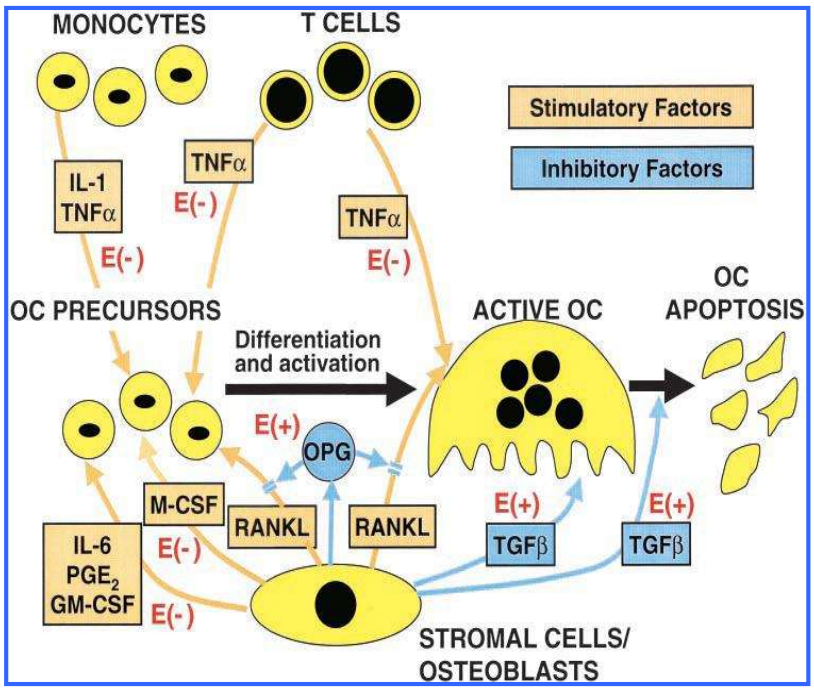
Two functional intracellular estrogen receptors proteins (ERs) namely ERα and ERβ are expressed in the osteoclasts, osteoblasts, bone marrow stromal cells and chondrocytes in the epiphyseal growth plate (Manolagas *et al*.,2002; Savendahl,2005). These receptors belong to the nuclear receptor gene superfamily. There is differential expression of ER in the growth plate and mineralized bone, with ERα more highly expressed in cortical than in cancellous bone and ERβ most evident at cancellous than cortical sites, suggests that they may have different functions. ERα appears to be the major receptor mediating estrogen action in bone, and it has a prominent effect on the regulation of bone turnover, maintenance of bone mass and growth promoting effect of estrogen (Nakamura *et al*.,2007; Figtree *et al*., 2009).

Effects of estrogen on BMD

The actions of estrogen are the major physiological mechanisms for bone mass conservation. Estrogen slows the rate of bone remodeling and protects against bone loss in several ways. First, by attenuating the birth rate of BMUs, which leads to lower bone turnover. Second, by increasing osteoclast apoptosis (shortening the resorption phase) and decreasing osteoblast apoptosis (prolonging the formation phase). Third, estrogen may affect the hormones that regulate calcium balance, calcitonin and PTH (Manolagas *et al*.,2002; Khosla,2007).

During the last decade, major progress has been made on elucidating the molecular mechanisms of estrogen action on bone cells. Local factors implicated in coordinating the sequence of events initiated by estrogen include IL-1,IL-6, prostaglandins (PGE-2), TNF, IGF system, OPG, TGFβ, GM-CSF, and M-CSF (Balasch,2003).

Estrogen regulates cellular activity by binding to ERs on the osteoblasts and it directly increases the production of OPG, and decreases the production of M-CSF, IL-6, PGE2 and GM-CSF (these cytokines increase bone resorption, mainly by increasing the pool size of preosteoclasts in bone marrow). Despite the presence of ERs in mature osteoclasts, it is possible that the binding of estrogen to receptors in osteoblasts regulates osteoclasts function, so the effect of estrogen on osteoclasts appears to be indirect (Papadopoulou and Krassas,2001).



E: estrogen
OC: osteoclast
Stimulatory factors are shown in *orange* and inhibitory factors are shown in *blue*. Positive (+) or negative (-) effects of E on these regulatory factors are shown in *red*.

Figure (6): Molecular mediators of Estrogen action on bone cells
(Riggs *et al*.,2002).

In addition to that, estrogen regulates RANKL indirectly by decreasing the secretion of the pro-inflammatory cytokines- IL-1 and TNFα by marrow monocytes and T-lymphocytes, and this results in

decreased production of RANKL by the osteoblasts, thereby decreasing the rate of production of osteoclasts, their activity and their survival. Estrogen also up-regulates TGF-β, an inhibitor of bone resorption that acts directly on osteoclasts to decrease their activity and increase rate of apoptosis (Riggs *et al*.,2002; Eastell,2005).

Menarcheal age and BMD:

There is evidence that age at menarche is more strongly related to BMD than age at menopause (Gerdhem and Obrant,2004). A late menarche is a determinant of BMD in the older woman and a strong risk factor for osteoporosis (Silman,2003).

There are two hypotheses that might explain this association. The first one relates to lifetime exposure to E2. Thus, a woman with an early menarche would have more years of exposure to E2 (Meyer *et al*.,1995; Eastell,2005). The second hypothesis assumes a postulated genetic cause of low PBM which is more plausible than shorter E2 exposure (Pérez-López *et al*.,2010).

Androgens

Testosterone is the major circulating androgen, secreted by the testes, can directly act on its receptor, the androgen receptor (AR) which are present in bone cells or it can also be converted locally to its more potent metabolite, dihydrotestosterone (DHT) by 5α-reductase. In addition, testosterone can act indirectly through the ERs via aromatization to E2 by aromatase, which occurs in bone tissues and in peripheral tissues, mainly in adipose tissues (Wiren,2005).

The adrenal glands secrete C19 androgens including dehydroepiandrosterone (DHEA) that can also be converted to E1 and E2

by aromatase, 17β-hydroxysteroid dehydrogenase (17β-HSD) and 3β-HSD or to testosterone by 17β-HSD and/or 3β-HSD (Meier and Kraenzlin ME,2007).

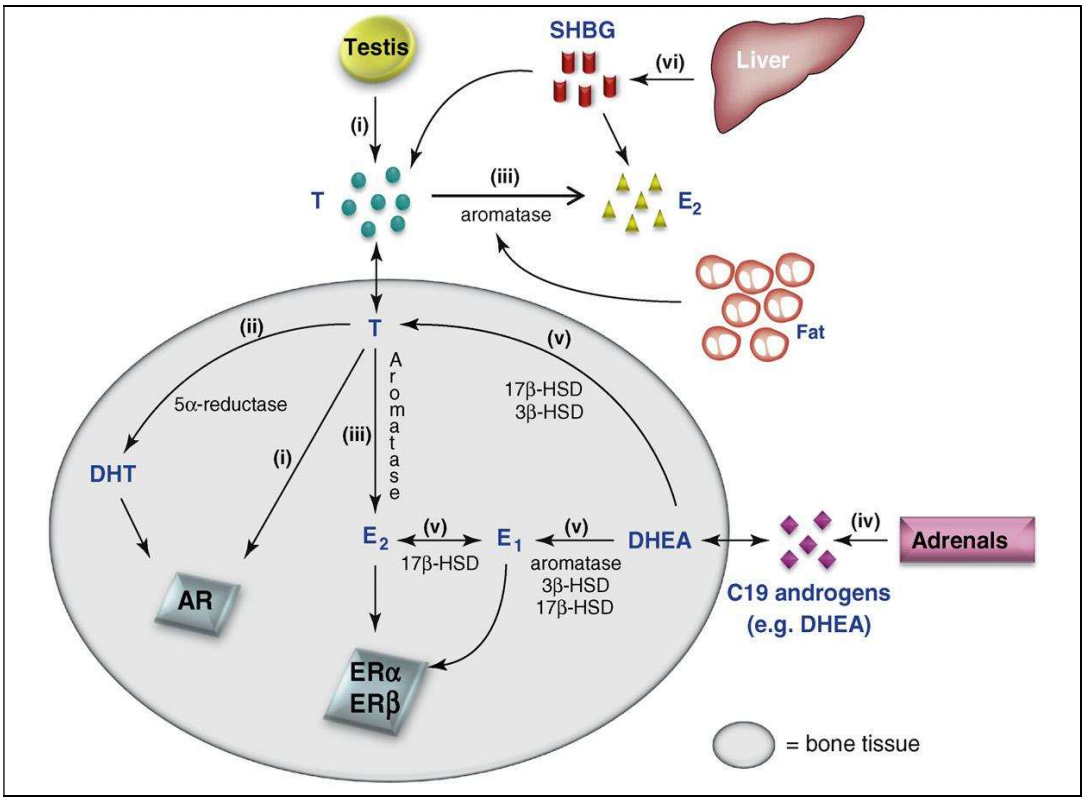


Figure (5): Metabolism of androgens (Callewaert *et al.*,2009).
T-testosterone

In fact, in men only about 20% of E2 is directly secreted by the testes, whereas the other 80% is derived from aromatization in peripheral tissues (Chiang,2009). There are reports of rare male patients with aromatase deficiency or ER defects; these subjects have a phenotype that includes tall stature and normal secondary sexual characteristics. However, these patients have osteoporosis and skeletal immaturity in adulthood despite normal androgen levels. Treatment of such patients with estrogen resulted in dramatic improvement in BMD and completion of skeletal maturation, indicating the critical role of aromatization of androgens into estrogens at the local level in maintaining normal bone remodeling in males (Hofbauer and Khosla,1999).

Androgens and their effects on bone

Androgens at the onset of puberty are responsible for the development of sexually dimorphic skeleton and for the production of PBM in males, so they are significant determinants of bone strength in males (Wiren,2005). There are several lines of evidence suggesting that androgens may also have indirect effects on calcium homeostasis by regulating the intestinal absorption and renal handling of calcium (Peacock,2010). As with estrogen, the major action of androgen is to reduce bone resorption. Nevertheless, much of this action is indirect via aromatization of androgen to estrogen. ARs are expressed in osteoblasts, osteocytes, chondrocytes, mononuclear cells and endothelial cells of blood vessels in the bone marrow as well as in the osteoclasts (Compston,2001).

Androgen increases the lifespan of osteoblasts by inhibiting their apoptosis, and has a modest effect on stimulation of osteoblast proliferation and differentiation. So, both effects of androgen contribute to its action on enhancing bone formation. Androgen action on the growth plate is clearly mediated via aromatization to estrogens and interaction with ER. Moreover, the action of androgens on osteoclasts by inhibiting their formation and/or function, remains controversial. (Callewaert *et al*.,2010).

Less is known about the molecular mechanisms of androgen action on bone cells, but many studies showed that its effects on stimulating osteoblast proliferation and differentiation are regulated by autocrine and paracrine factors in the bone microenvironment, including TGF- β, IGF-I, IGF-II, and IL-6 (Balasch,2003), while the effects of androgens on inhibiting bone resorption may be mediated, at least in part, by increased TGF-β production and activity, decreased IL-6 production, and this inhibition is similar to that achieved with estrogen (Riggs *et al*.,2002).

On the other hand, androgen differs from estrogen, in that, androgen decreases OPG production, while estrogen increases it, which may partly explain why the antiresorptive action of androgen is weaker than that of estrogen, so androgen levels are likely to be less significant determinant of bone resorption than those of estrogen (Wiren,2005).

Bone mineral density (BMD)

Bone mineral density (BMD) is a medical term referring to the amount of matter (mineral) per square centimeter of bones (g/ cm^2). Epidemiological studies have consistently shown that BMD is the most commonly used outcome measure to address skeletal status and it is a primary predictor of osteoporosis and fracture risk (Barclay *et al*.,2006).

Skeletal mass increases from approximately 70 and 95 g at birth to 2400 and 3300 g in young women and men, respectively. Most of the increase in BMD occurs during the pubertal growth spurt, although small increases in BMD continue for an undefined interval after linear growth ceases, by a process termed bone consolidation (Dent,1999). Different studies have shown that approximately 40% of bone mass is accumulated during adolescence (Theintz *et al*.,1992; Bailey *et al*.,1999).

This dramatic accumulation of bone mass during puberty is caused by changes in both modeling and remodeling that occur simultaneously during this period of life in a way that both bone deposition and bone mass formation exceed resorption, leading to increased BMD that coincides with accelerated weight and height growth (Doneray and Orbak,2008; De Andrade.,2010).

peak bone mass (PBM)

BMD increases during childhood and adolescence until the PBM is achieved, PBM is defined as the maximal BMD that is accrued during

growth and development plus subsequent consolidation that continues during early adulthood (Sluis *et al*.,2002; Davies *et al*.,2005). PBM is regarded as the bone bank for the remainder of life (Mora and Gilsanz,2003). The process of "consolidation" brings the skeleton to its maximal values by continued periosteal apposition and, possibly, also by trabecular thickening. How long consolidation continues is disputed, some argue that it lasts only until the end of the second decade, whereas others argue that it may last until the end of the third decade (Avdagic *et al*.,2009).

After the PBM is achieved, bone mass stabilizes and then decreases progressively in both sexes after 35-40 years of age with a steeper decline in women after the menopause (Lu *et al*.,1994).

Determinants of BMD :

Many factors, more or less dependent on each other, are known to influence bone mass accumulation during growth:

• **Genetics**- Twin studies showed a higher heritability, up to 80%, this might be an overestimation as a result of more common lifestyle factors (Dequeker *et al*.,1987; Pocock *et al*.,1987), whereas the remaining 20% is modulated by environmental factors and sex hormone levels during puberty (Gueguen *et al*.,1995). This genetic influence is consistent with the findings that BMD is reduced in the daughters of osteoporotic women (Nguyen *et al*.,2003). A large number of genes are responsible for determining bone mass, and polymorphisms of many of these have been suggested to influence bone mass (Davies *et al*.,2005).

• **Ethnic differences-** BMD in Asians people is reported to be lower than other people (Pajouhi *et al*.,2004), and some researchers observed that black youths have greater bone mass than Whites (Gilsanz *et al*.,1991).

• **Gender differences-** The timing of BMD acquisition differs between males and females, which is apparently due to the different onset and progression of puberty according to sex steroids production and their effects on target tissues (Seeman,2008), and it has been suggested that ultimately these gender differences during adolescence at the spine disappear as boys catch up with puberty and growth (Bertelloni *et al*.,2010). In both sexes bone mass progressively increases during childhood, with a rapid gain during puberty. However, some gender differences in the accrual of bone mass are evident. At birth and during prepubertal period, males and females have similar values of BMD measured by DXA, then BMD values become higher in females than in males as the results of the earlier onset of the puberty, whereas in the late adolescence BMD in boys exceeds that measured in girls (Boot *et al*.,1997; Nguyen *et al*.,2001).

• **Physical activity-** Maximizing skeletal exposure to mechanical loading during growth appears to be an effective strategy to optimize bone accrual. Physical activity is essential for the correct development of bone. It is believed that muscular action transmits tension to the bone, which is detected by the osteocyte network within the osseous fluid. These osteocytes produce regulators such as prostaglandins and IGF-I, which stimulate the osteoblast activity and hence, increase bone formation.

On the other hand, inactivity poses a great threat to BMD gains, television viewing and computer use continue to increase, and the time spent in physical activities is declining. This habit seems to be more frequent in selected groups of youths (Havill *et al*.,2007; Weeks and Beck,2010).

• **Diet-** Good nutrition from infancy through adolescence, with particular attention to adequate daily intake of calcium and vitamin D, is a key component for the attainment of maximum PBM. Some studies have

shown an increased prevalence of osteoporosis in regions where dietary calcium intake is extremely low (Deepika *et al*.,2010).

• **Lifestyle factors-** A few trends are likely to manifest during adolescence, as increasing the use of tobacco and alcohol drinking; which are associated with a reduced BMD (Pérez-López *et al*.,2010).

• **Hormonal Influences on Bone Mass-** The endocrine system is highly involved in the regulation of bone metabolism and growth. During childhood, the major systemic hormones involved in skeletal development are: GH, IGF- I, estrogen and androgen (Balasch,2003).

Definition of osteoporosis and its prevention

Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture (Rasheed, 2008). Osteoporosis represents a major societal health burden; and although it has traditionally been considered a disease of the elderly, it has its origins during childhood due to impaired PBM acquisition. This has led to increasing interest in assessing BMD in children and adolescents (Wren and Gilsanz,2006; Talwar *et al*.,2007). Although genetic influences are the predominant determinant of BMD, it is only environmental influences which may be modified to optimize bone mass in childhood and adolescence by promoting a healthy lifestyle, including physical activity and diet (Sheth *et al*.,1996).

Weight-bearing activity should be encouraged, and even short periods of high intensity exercise (such as running, jumping, gymnastics) for 10-20 minutes, at least 3 days per week. Calcium intake should meet the recommended daily intake of 1300 mg for children and adolescents

from` 9-18 years of age, and daily vitamin D supplementation of 200 IU or more (Davies *et al*.,2005 Barclay *et al*.,2006).

Dual-Energy X-ray Absorptiometry (DXA)

BMD is measured by a procedure called densitometry, in order to identify patients at greatest risk of skeletal fragility fractures, to guide decisions regarding treatment, and to monitor responses to therapy (Gilsanz and Wren,2007).

Several noninvasive imaging techniques are currently available to assess pediatric skeleton. The most commonly used densitometric technique throughout the world is DXA which was developed in the late 1980s and is now widely available due to its precision, short scan times, very low radiation exposure, painlessness, low cost and robust pediatric reference data (Lazaretti-Castro, 2004).

Effective dose of DXA of the lumber spine is 0.4-4 μSv (micro sievert), compared with the dose of chest x-ray 12-20 μSv (Fewtrell,2003), so, the exposure to this very low dose of ionizing radiation with DXA poses no known health risk (Bachrach *et al*.,2011). Using x-ray beams at 2 photon energies, DXA determines the amount of mineral in a given region; it relies on the differential absorption of x-rays to distinguish tissues of different radiographic density (Binkovitz *et al*.,2007).

DXA examination generates numeric results and diagnosis, thus it is different from all other imaging studies. It can quantify the bone mineral content (BMC; in grams) at various body sites; a bone area is delineated (units of cm^2), and the BMD is measured directly (in units of g/ cm^2, by dividing BMC on bone area). The DXA-derived BMD is based on the 2-dimensional projected area of a three dimensional structure because the third dimension, depth, cannot be identified in that it has the same

direction as the x-ray beam. Therefore, BMD is an areal, rather than volumetric density, and the results are a sum of cortical and trabecular BMC within the projected bone area (Pérez-López *et al*.,2010). As with other laboratory tests, the numeric value reported is meaningless without comparison with the appropriate normal controls matched for age, sex, height, weight and ethnicity (Loud and Gordon,2006). This comparison is reported as a standard deviation score, the Z-score. If a BMD Z-score is less than or equal to -2; it should be labeled as "low BMD for chronologic age". The terms "osteopenia" and "osteoporosis," which are used to describe milder or greater deficits in BMD in older adults, should not be used for pediatric patients. The T-score is a standard deviation score, compared the reported BMD value with peak adult BMD, it is used in adult interpretation but should not be included in the pediatric DXA report (Binkovitz *et al*.,2007; Gordon *et al*.,2008).

Bone densitometry is considerably more difficult to perform in growing children than adults. Immobilization is more difficult in children, thus the number of skeletal sites which can be scanned successfully may be limited. The posterior anterior (PA) lumber spine is a preferred site in pediatrics, because of its speed and precision of measurement, easily identified bony landmarks, increasing amounts of pediatric normative data, and the abundance of trabecular bone in the lumber spine that more accurately reflect the rate of bone turnover and therefore approximates alterations in mineralization. In addition to that, it is the possibility to perform serial measurements at precisely the same localization (L_1-L_4) (Khan *et al*.,2004; Bachrach *et al*.,2011).

The Pediatric Position Development Conference of the International Society of Clinical Densitometry guidelines identified the disorders that have been associated with evidence of increased fracture risk (table-2).

Table (2): Diseases Associated with Low Bone Mass or Fractures in Children and Adolescents (Soyka *et al*.,2000; Lim,2010; Bachrach *et al*.,2011).

Genetic Defects	Osteogenesis imperfecta Turner's syndrome (XO) Klinefelter's syndrome (XXY) Down's syndrome (21 trisomy) Marfan syndrome Phenylketonuria Glycogen storage disease Wilson disease Cystic fibrosis Heredity hemochromatosis	Endocrine disorders	Diabetes Hypogonadism GH deficiency Primary hyperparathyearoidism Cushing's syndrome
		Nutritional Disorders	Malnutrition Anorexia Nervosa Preterm infants Vitamin D deficiency Vitamin K deficiency Calcium deficiency
Chronic diseases	Rheumatic disorders (juvenile rheumatic arthritis , systemic lupus erythromatosis) Renal disease Inflammatory bowel disease Malabsorption (celiac disease) Liver disease Congenital heart disease Chronic obstructive lung disease Hemophillia Leukemia, Lymphoma Solid tumors	Disorders causing disuse osteoporosis	Chronic diseases Cerebral palsy Huntington disease Muscular dystrophy Burns
		Iatrogenic disorders	Glucocorticoid excess Immune suppressant Anticonvulsants Chemotherapy Radiotherapy
		Others	Idiopathic juvenile osteoporosis Constitutional delay of puberty

Aims of the study

This study aims:

» To determine the changes in BMD, growth parameters [weight, height and body mass index (BMI)], calcium, phosphorus, ALP and sex steroids in healthy pubertal males and females from Mosul city/Iraq at different pubertal stages.

» To allow an insight into gender dimorphism in mineral accretion during puberty. This study provides gender-specific lumber spine BMD values (measured by DXA), expressed in discrete age and pubertal stage subgroups.

» To detect the relationship of BMD with growth parameters, calcium, phosphorus, ALP and sex steroids, and to reveal the most important determinant of BMD in the pubertal period.

Subjects, Materials and methods

Subjects

This study represents a case series study, carried out from November 2010 to March 2011. The participants were (177) healthy children and adolescents, including (96) males and (81) females, aged (10-20.2) years, residents of Mosul city/Iraq, they were selected from different sources:

- Outpatient clinic at Ibn-Sina Hospital, where healthy subjects who accompanied the patients were invited into the study.
- Relatives of the staff members in Ibn-Sina Hospital.
- Students at the 1st class in the College of Medicine/ Mosul University and students at the nursing secondary school.

These subjects were invited to take part in this study and interviewed with their parents to explain the methods used and seek oral consent. A questionnaire (appendix-1) was administered to all subjects and their families.

Exclusion criteria : To carry out the study; the following exclusion criteria were used to choose the subjects:

- Subject with any chronic disease that is associated with low bone mass, which are listed in (table-2).
- Drug consumption- as corticosteroids, anticonvulsants, calcium and vitamin supplements, antacids, immune suppressant drugs (Henwood *et al*.,2009; Bachrach *et al*.,2011).
- Doing exercises in a professional manner, especially the physical training sessions requiring jumping (8-10 hours training sessions a week) (Bozkurt,2010), since investigations indicate that programmed sport

activities demonstrate the greatest increases in bone mass (MacKelvie *et al*.,2002).

- Subjects who were below the 5th percentile or above 95th percentile for weight, height and BMI were excluded (appendix-2)(Hamil *et al*.,1979).

- Females with irregularity of menstrual periods, a history of menstrual irregularities has been consistently associated with lower BMD in premenopausal women (Balasch,2003).

- A significant fracture history- PDC of the International Society of Clinical Densitometry has defined a clinically significant fracture history as "one long bone fracture of the lower extremity, two or more long bone fractures of the upper extremity or a vertebral fracture (Bachrach *et al*.,2011).

- Smoking which has been linked to low BMD in adolescents (Henwood *et al*.,2009; Pérez-López *et al*.,2010).

- Family history of osteoporosis, there are evidences for a strong familial resemblance of BMD (Dequeker *et al*.,1987; Pocock *et al*.,1987).

Anthropometry

Body weight was measured with an electronic scale with the subjects wearing light clothes without shoes. Height was measured using a vertical scale while the subject stand erect without shoes. Both weight and height were derived using growth curves published by the U.S. National Center for Health Statistics. BMI was calculated by using the following formula (Guyton and Hall,2006):

BMI (kg/m^2) = weight (in kilogram) / height^2 (in meters)

Pubertal staging

According to the method of Tanner, pubertal development was evaluated by self-assessment of breast and pubic hair for girls and genitalia and pubic hair for boys. Subjects were given pictures and written descriptions (appendix-3), and selected the picture that most accurately reflected their appearance. Self-assessments are nonetheless used in many bone density centers, especially when a full examination is not possible (Gordon *et al*.,2008).

In addition to self assessment, examination of female subjects (breast development) has been done by the researcher. On the other hand, a help of pediatrician was asked to examine male subjects, when there was any discrepancy especially between stage III and IV. Then subjects were classified as Tanner stage I, II, III, IV, and V (Shirtcliff *et al*.,2009).

Measurement of BMD

Lumber spine (L_1-L_4) BMD expressed in g/ cm^2 was measured with DXA scans using Hologic /Discovery (W-S/N 83903,USA).

To measure BMD of the lumber spine with a DXA machine, the person lies supine on a flat padded table with elevation of the knees in order to flatten the physiological lumber lordosis, the person remains motionless and the "arm" of the instrument passes over the selected areas. While the measurement is performed, a beam of low dose x-rays from below the table passes through the area being measured (figure-7). These x-rays are detected by a device in the instrument's arm.

The machine converts the information received by the detector into an image of the skeleton and analyzes the quantity of bone that skeleton contains. The results are usually reported as BMD, the amount of bone

per unit of skeletal area (appendix-4). DXA equipment was calibrated using a lumbar spine phantom as recommended by the manufacturer.

Materials: Materials utilized through out this study were arranged as follow:

-Specimen
-Reagents
-Instruments

Specimens:

Five ml of blood was drawn in non-fasting state between 9-11 Am, using anti-cubital venepuncture without tourniquet. Blood samples were drawn by a skilled nurse. The samples incubated at 37°C for 15 minutes in water bath, then centrifuged at 3000 rpm (rotations per minute) for 15 minutes to insure good separation. Serum samples were collected in two separated plain tubes and stored at -20°C until assayed. Before conducting the assay, thawing of samples was allowed to take place at 4° C.

For the females who began menstruation (their number=44), the blood samples were collected between 2-5 days after menstrual bleeding started, in order to measure serum estradiol (E_2) during early follicular phase.

With the aim of minimizing drawbacks in the present study, all adolescents were measured under the same conditions. Blood samples were collected and stored at the same time in the morning. All adolescents were measured from November 2010 to March 2011 at stable temperature inside the hospital.

Reagents:

The selection of reagents used in this study was based on accuracy, reliability and availability. They were supplied from different companies and will be described in details within the related methodology.

Instruments:

All biochemical analyses were performed in the laboratory of Ibn-Sina teaching hospital. The following instruments were used throughout this study:

Water bath (Memert, Germany).

Centrifuger (Kokusan, H-19F, Japan).

Incubator (Fisher,USA).

Spectrophotometer (Cecil CE1011, UK).

Minividas (Bisemesis, France) (figure-8).

Methods:

Markers of the mineral economy of the bone were conducted for each subject including serum calcium and phosphorus, while serum total ALP was measured as a marker of bone turnover. Serum testosterone and serum E_2 were measured for male subjects and female subjects, respectively; both parameters reflect the sex steroid hormone state of the subject, which affects bone turnover.

Measurement of serum total calcium (mmol/l) :

Total calcium in serum sample was measured colorimetrically (Burtis *et al*.,2008) by a kit supplied from Biolabo SA, France.

Principle: In a weak acidic media, metallo-chromogen Arsenazo III combines with calcium to form a colored complex, their absorbance (Abs) measured at 650 nm.

Reagents:

R1 Arsenazo III reagent	Imidazol buffer pH 6.8 at 25˚C	> 90 mmol/L
	Arsenazo III	> 0.18 mmol/L
	Surfactant	0.1 %
	Preservative	
R2 standard	Calcium	10 mg/dl (2.5 mmol/L)

Procedure: Pipette into well identified test tube:

	blank	standard	assay
Reagent	1 ml	1 ml	1 ml
Distilled water	20µl	_	_
Standard	_	20µl	
Specimen	_	_	20µl
Mix well. Let stand for 1 minute at room temperature. Read abs at 650 nm against reagent blank. The coloration is stable for 1 hour.			

Calculation:

$$\text{Sample concentration (mmol/l)} = \frac{\text{Abs of sample}}{\text{Abs of standard}} \times \text{concentration of standard}$$

Calculation of corrected calcium:

Because albumin is the principle binding protein for calcium, a fall in the plasma concentration of albumin will lead to a fall in bound calcium and a decrease in total calcium concentration (and vice versa). So various calculations have been used to correct total calcium for variation in protein concentration; one of them is the following formula (Smith *et al*.,1989), which was used in this study :

Corrected calcium = measured calcium+0.02 (40- albumin)

(mmol/l) (mmol/l) (g/l)

Measurement of serum albumin (g/l):

Serum Albumin was determined by using a dye-binding method (Burtis *et al*.,2008), using kit supplied from biomerieux , France.

Principle: Albumin-kit enables colorimetric determination of albumin in human serum or plasma. At pH 4.2 albumin combines with bromocresol green to form a blue-green complex

$$\text{Albumin+bromocresol green} \xrightarrow{\text{pH 4.2}} \text{albumin-bromocresol green complex}$$

The intensity of the complex coloration is proportional to the quantity of albumin present in the sample.

Reagents:

R1 Standard	R1	Bovine albumin Sodium merthiolate	714.5 µm/l 0.19 g/l
R2 Color reagent	R2	Bromocresol green Succinate buffer pH 4.2 Brij 35 Sodium merthlotate	0.23 mmol/l 75 mmol/l 2.1 g/l 0.1 g/l

Procedure:

Pipette into appropriate tubes as follows:

	Reagent blank	Standard	Sample
Standard	_	10 µ	_
Sample	_	_	10 µl
R2	2.5 ml	2.5 ml	0.5 ml
Mix. Leave for 5 minutes at 20-25ºC. Perform photometry.			

Read abs against zero adjusted reagent blank at 628 nm.

Calculation:

$$\text{Sample concentration (g/l)} = \frac{\text{Abs of sample}}{\text{Abs of standard}} \times \text{Concentration of standard}$$

Measurement of serum inorganic phosphorus (mmol/l):

Phosphorus in serum sample was measured colorimetrically (Burtis *et al*.,2008) by a kit supplied from Biolabo SA, France.

Principle: In an acid medium, phosphate ion forms a phosphomolybdic complex with the ammonium molybdate. The abs was measured at 340 nm.

Reagents:

R1 molybdate reagent	ammonium molybdate	0.63 mmol/l
	sulfuric acid	210 mmol/l
	surfactant	
R2 standard	Phosphorus	5 mg/dl (1.61 mmol/l)

Procedure: Into the following tubes add:

	blank	Specimen blank	standard	assay
Reagent	1ml	_	1ml	1ml
Saline solution	_	1ml	_	_
Demineralised water	20µl	_	_	_
Standard	_	_	20µl	_
Specimen	_	20µl	_	20µl
Mix well, incubate for 2 minutes at room temperature. Read standard and assay abs at 340 nm against reagent blank. Read specimen blank against saline solution.				

Calculation:

$$\text{Sample concentration (mmol/l)} = \frac{\text{Abs (assay)} - \text{Abs (specimen blank)}}{\text{Abs (standard)}} \times \text{concentration of standard}$$

Measurement of serum total ALP activity (IU/l):

Serum ALP activity was measured colorimetrically (Belfeild and Goldberg,1971) by a kit supplied from Biomerieux, France. Bone specific ALP was not available at time of the study, so total ALP had been measured.

Principle: colorimetric determination of activity was considered according to the following reaction:

$$\text{Phenylphosphate} \xrightarrow[\text{pH 10}]{\text{Alkaline phosphatase}} \text{phenol} + \text{phosphate}$$

The librated phenol is measured in the presence of 4-aminoantipyearine and potassium ferricyanide. The presence of sodium arsenate in the reagent stops the enzymatic reaction.

Reagents:

R1 substrate buffer	Disodium Phenylphosphatase Carbonate-bicarbonate Buffer pH 10 Sodium meriolate	5 mmol/l 50 mmol/l 0.1 g/l
R2 standard	Phenol	20 Kind and King U (142 U/l)
R3 blocking reagent	4-aminoantipyearine sodium arsenate	60 mmol/l 75 g/l
R4 color reagent	potassium ferricyanide	150mmol/l

Procedure:

Set up the following tubes:

	Serum sample	Serum blank	standard	Reagent blank
Reagent 1	2 ml	2 ml	2 ml	2 ml
Incubate for 5 minutes at 37˚C.				
serum	50µl	_	_	_
Reagent 2	_	_	50µl	_
Incubate for exactly 15 minutes at 37˚C.				
Reagent 3	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Mix well or preferably vortex				
Reagent 4	0.5 ml	0.5 ml	0.5 ml	0.5 ml
serum	_	50µl	_	_
Distilled water	_	_	_	50µl
mix. let stand for 10 minutes in the dark.				

Then, measure the abs of serum sample, serum blank, and standard against zero blank using a wave length of 510 nm.

Calculation:

$$\text{Sample concentration (IU/l)} = \frac{\text{Abs Serum sample} - \text{Abs Serum blank}}{\text{Abs Standard}} \times \text{Concentration of standard}$$

Measurement of serum testosterone (ng/ml)

Testosterone concentration was measured by Minividas, using Enzyme Linked Fluorescent Assay technique (ELFA), the kit is supplied from Biomerieux (France). The detection limit of this method is <0.1 ng/ml and the measurement range is 0.1-13 ng/ml (Litwack,1992).

Principle: The assay principle combines of an enzyme immunoassay competition method with a final fluorescent detection (ELFA). The solid phase receptacle (SPR) represents the solid phase as well as the pipetting device for the assay. The SPR is coated with anti-testosterone specific antibody. The reagents for the assay are located in the sealed reagent strips. The sample is transferred into the well containing the anti-testosterone antibody conjugated with ALP. The sample/conjugate mixture is cycled in and out of SPR several times. The testosterone present in the serum and the testosterone derivative in the conjugate compete for anti-testosterone specific antibody sites coated to the inner surface of SPR, so the testosterone will bind to the antibodies and to the conjugate forming a sandwich. Unbound components are eliminated during the washing steps.

During the final detection step, the substrate (4 methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of substrate into fluorescent product (4-methyl umbelliferone), the fluorescence of which is measured at 450nm. The intensity of the fluorescence is inversely proportional to the concentration of testosterone present in the sample. At the end of the assay, results are automatically calculated and printed out by the instrument in relation to the calibration curve stored in the memory.

Content of the kit

Testosterone strips	STR	Ready to use
Testosterone SPRs	SPR	Ready to use. SPRs sensitized with polyclonal anti- testosterone immunoglobulins (rabbit).
Testosterone control (lyophilized)	C1	Reconstitute with 2 ml of distilled water. Wait for 5 to 10 minutes. Mix after reconstitution.
Testosterone calibrator (lyophilized)	S1	Reconstitute with 3 ml of distilled water. Wait for 5 to 10 minutes. Mix after reconstitution.

Description of testosterone reagent strip

Wells	Reagents		
1	Sample well		
2-3-4	Empty well		
5	Conjugate: ALP-labeled testosterone derivative +calf serum + gelatine (porcine) + releasing agent + 0.9 g/l sodium azide (400µl)		
6	Empty well		
7-8	Wash buffer	Tris-NaCl pH 7.4	0.05 mol/l
		Sodium azide (600µL)	0.9 g/l
9	Wash buffer	Diethanolamine pH 9.8	1.1 mol/l or (11.5%)
		Sodium azide (600µL)	1 g/l
10	Cuvette with 4 methyl umbelliferyl phosphate		0.6 mmol/l
	Diethanolamine pH 9.2		0.62 mol/l or(6.6%)
	Sodium azide (300µL)		1g/l

Procedure: the procedure for measuring testosterone used in this study involves the following:

1. Using a vortex type mixer, mix calibrator, control and samples.
2. Using one strip and one SPR for each calibrator, control or sample.
3. Adding 200µL of calibrator, control or sample into sample well.
4. Making sure that the color labels with the assay code on the SPRs and strips matched.
5. Typing or selecting testosterone to enter the test code. The calibrator must be identified by S_1 and tested in duplicate, if the control is to be tested, it should be identified by C_1.
6. All assay steps were performed automatically and completed within 60 minutes.
7. The results were analyzed automatically from the calibration curve, and printed out, all expressed in ng/ml.
8. At the end of the assay, the strips and SPRs were removed and disposed into an appropriate place.

Measurement of serum estradiol (E2)(pg/ml):

Serum E_2 concentration in the serum was measured by Minividas, using Enzyme Linked Fluorescent Assay technique (ELFA), the kit is supplied from Biomerieux (France). The detection limit of this method is <9 pg/ml and the measurement range is 9-3000 pg/ml (Litwack,1992).

Principle: The assay principle combines of an enzyme immunoassay competition method with a final fluorescent detection (ELFA). The SPR represents the solid phase as well as the pipetting device for the assay. The SPR is coated with anti-E_2 specific antibody. The reagents for the assay are located in the sealed reagent strips. The sample is transferred into the well containing the anti-E_2 antibody conjugated with ALP. The sample/conjugate mixture is cycled in and out of SPR several times. The E_2 present in the serum and the E_2 derivative in the conjugate compete for anti-E_2 specific antibody sites coated to the inner surface of SPR, so the E_2 will bind to the antibodies and to the conjugate forming a sandwich. Unbound components are eliminated during the washing steps.

During the final detection step, the substrate (4 methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of substrate into fluorescent product (4-methyl umbelliferone), the fluorescence of which is measured at 450nm. The intensity of the fluorescence is inversely proportional to the concentration of E_2 present in the sample. At the end of the assay, results are automatically calculated and printed out by the instrument in relation to the calibration curve stored in the memory.

Content of the kit

E_2 strips	STR	Ready to use
E_2 (SPRs)	SPR	Ready to use. SPRs sensitized with polyclonal anti- E_2 immunoglobulins (rabbit).
E_2 control (liquid)	C1	Ready to use. Human serum+17β E_2+ 1g/l sodium azide.
E_2 calibrator (liquid)	S1	Ready to use. Human serum+17β E_2+ 1g/l sodium azide.

Description of reagent strip

Wells	Reagents		
1	Sample well		
2-3-4	Empty well		
5	Conjugate: ALP-labeled E_2 derivative + 0.9 g/l sodium azide (400µl)		
6	Empty well		
7-8	Wash buffer	Tris-NaCl pH 9	0.05 mol/l
		Sodium azide (600µL)	1g/l
9	Wash buffer	Diethanolamine pH 9.8	1.1 mol/l or (11.5%)
		Sodium azide (600µL)	1 g/l
10	Cuvette with 4 methyl - umbelliferyl phosphate		0.6 mmol/l
	Diethanolamine pH 9.2		0.62 mol/l or(6.6%)
	Sodium azide (300µL)		1g/l

Procedure: the procedure for measuring E2 used in this study involves the following:

1. Using a vortex type mixer, mix calibrator, control and samples.
2. Using one strip and one (SPR) for each calibrator, control or sample.
3. Adding 200µL of calibrator, control or sample into sample well.
4. Making sure that the color labels with the assay code on the SPRs and strips matched.
5. Typing or selecting E2 to enter the test code. The calibrator must be identified by S_1 and tested in duplicate, if the control is to be tested, it should be identified by C_1.
6. All assay steps were performed automatically and completed within 60 minutes.
7. The results were analyzed automatically from the calibration curve, and then printed out, all expressed in pg/ml.
8. At the end of the assay, the strips and SPRs were removed and disposed into an appropriate place

Figure (7): Dual energy x-ray absorptiometry (DXA) machine (Hologic /Discovery W-S/N 83903,USA).

Figure (8): Minividas (Bisemesis, France).

Statistical analysis

All analyses were carried out by using the Statistical Package for the Social Science (SPSS Inc, Chicago, version 11.5).

- As descriptive statistics, mean and standard deviation (SD) were given for the data. Analyses were performed for males and females separately; differences between the two groups were assessed by unpaired *t*-test.
- The effects of puberty on the parameters and the effects of age on the BMD values were assessed using one-way analysis of variance (ANOVA), followed by Duncan´s multiple range tests for each gender separately.
- Pearson's correlation coefficient was used to find the relationships between BMD and the other parameters, these correlations were represented as scatter diagrams.
- The association between BMD and different predictor variables was tested with stepwise multiple regression analysis.
- Differences between observations were considered statistically significant at $p<0.05$, whereas they were considered highly significant at $p<0.001$ (Daniel,1999).

Results

The demographic characteristics of the study:

Number and percentage of subjects in each pubertal stage:

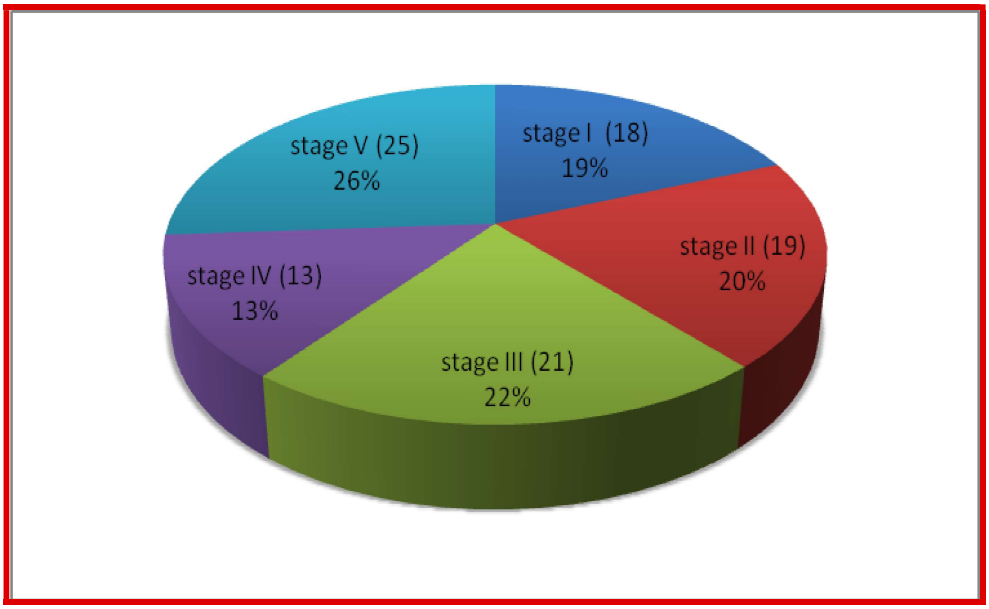
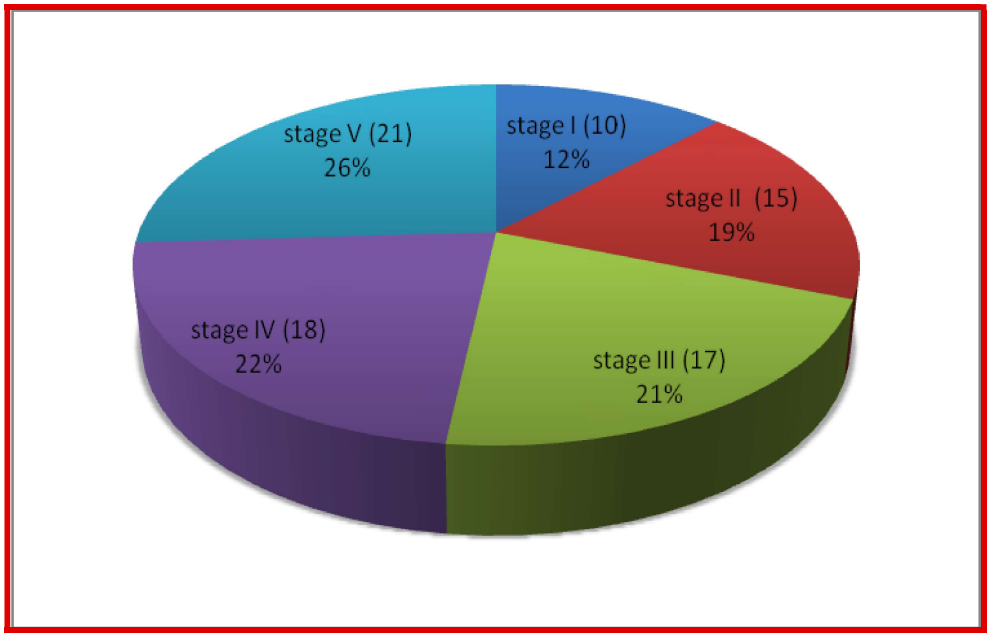


Figure (9): Number and percentage of males in each pubertal stage
Stage (number)- percentage.



Figure(10): Number and percentage of females in each pubertal stage
Stage (number)- percentage.

Number and percentage of subjects in each age group:

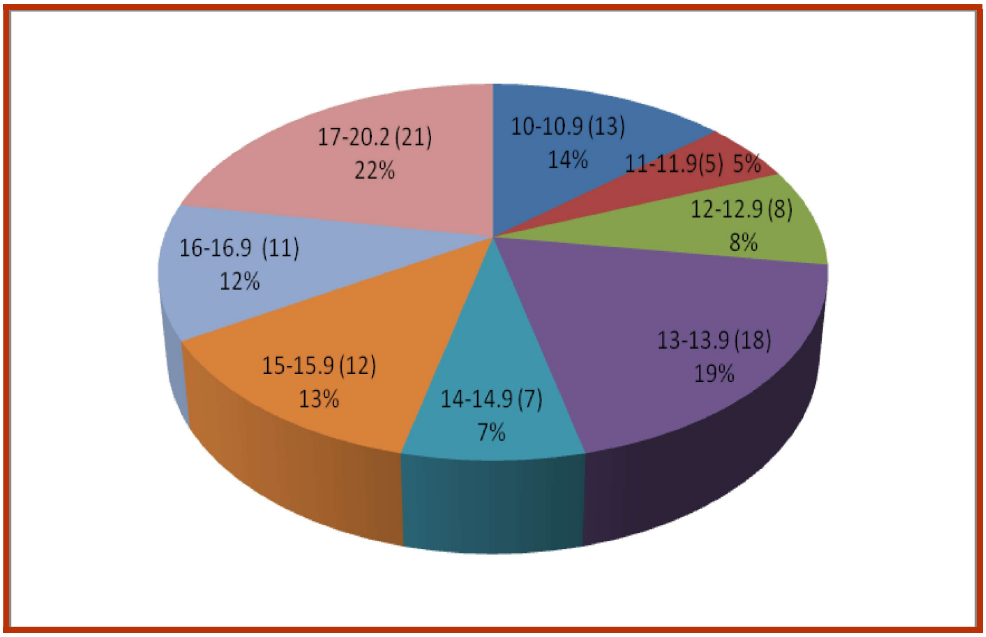


Figure (11): Number and percentage of males in each age group
Age group (number)- percentage.

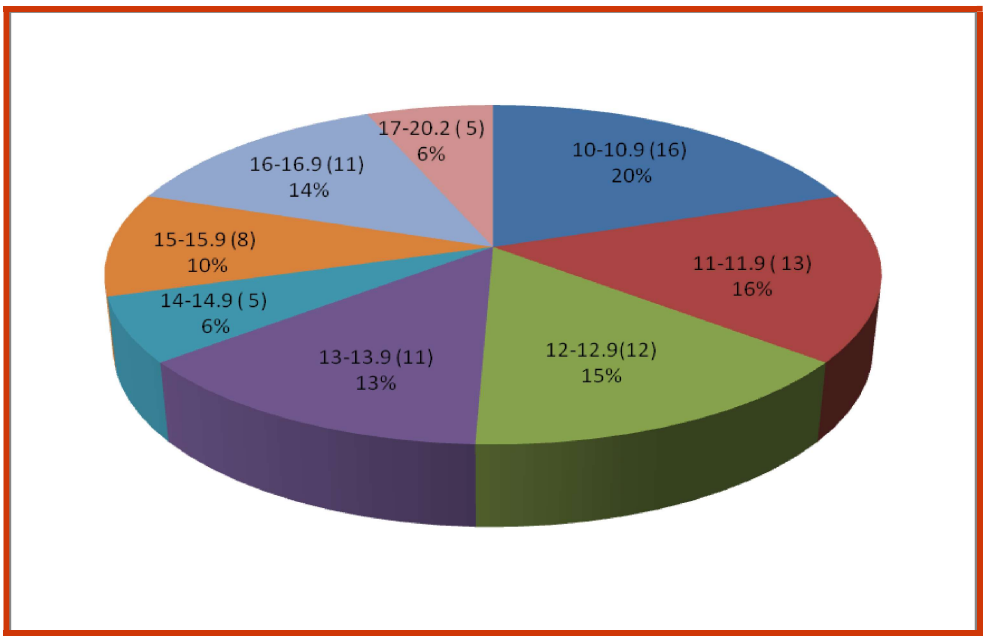


Figure (12): Number and percentage of females in each age group
Age group (number)- percentage.

Table (2): Comparison of the anthropometric and clinical characteristics between males and females

Parameters	Male (n=96) mean±SD	Female (n=81) mean±SD	p-value
Age (year)	14.4±2.8	13.4±2.8	<0.05*
Weight (Kg)	51.2±14.9	43.6±10.3	<0.001**
Height (cm)	157.38±11.4	150±8	<0.001**
BMI (Kg/m^2)	20.3±3.8	19.2±3.1	<0.05*
BMD (g/cm^2)	0.719±0.17	0.723±0.16	0.866 (NS)
Calcium (mmol/l)	2.36±0.1	2.38±0.1	0.114 (NS)
Phosphorus (mmol/l)	1.42±0.6	1.55±0.6	0.141 (NS)
ALP (IU/l)	169.5±81.4	166.2±78	0.64 (NS)

* *means significant difference, p-value<0.05*

** *means highly significant difference, p-value<0.001*

NS means no significant difference.

Table (2) compares the parameters between both sexes in the studied group by using unpaired *t*-test. The mean age of the subjects was 14.4±2.8 years for males and 13.4±2.8 years for females. As anticipated, males were significantly taller, heavier and with greater BMI. It seemed that both sexes had no significant differences regarding BMD, calcium, phosphorus, and ALP.

Comparison of parameters between males and females in each pubertal stage:

Analyses were performed for each pubertal stage (I to V) for males and females separately and then differences between the two groups in each stage were assessed by unpaired *t*-tests (tables 3-7):

Table (3): Comparison of parameters between males and females in pubertal stage I

Parameters	Male (n=18) mean±SD	Female (n=10) mean±SD	p-value
Age (year)	10.98±1.4	10.4±0.99	0.272 (NS)
Weight (Kg)	33.6±5.4	32.67±5.54	0.673 (NS)
Height (cm)	142.39±5.16	140.11±6.99	0.346 (NS)
BMI (Kg/m^2)	16.53±2.2	16.53±1.5	0.997 (NS)
BMD (g/cm^2)	0.518±0.03	0.512±0.04	0.673 (NS)
Calcium (mmol/l)	2.32±0.1	2.36±0.1	0.44 (NS)
Phosphorus (mmol/l)	2±0.74	2.03±0.7	0.921 (NS)
ALP (IU/l)	228.72±58.4	186±61.9	0.097(NS)

NS means no significant difference.

Table (3) demonstrated that the subjects were in stage I which is regarded as prepubertal stage. There were no significant differences between males and females in all parameters (age, body weight, height, BMI, BMD, calcium, phosphorus, and ALP).

Table (4): Comparison of parameters between males and females in pubertal stage II

Parameters	Male (n=19) mean±SD	Female (n=15) mean±SD	p-value
Age (year)	12.66±1.5	11.5±1.38	<0.05*
Weight (Kg)	43.6±8.09	37.53±6.14	<0.05*
Height (cm)	150±7.2	145.8±6.43	0.087 (NS)
BMI (Kg/m^2)	19.3±3	17.6±1.9	0.056 (NS)
BMD (g/cm^2)	0.598±0.03	0.583±0.03	0.165 (NS)
Calcium (mmol/l)	2.32±0.08	2.34±0.1	0.554 (NS)
Phosphorus (mmol/l)	1.68±0.66	1.97±0.58	0.19 (NS)
ALP (IU/l)	250.2±50.6	220.93±64.2	0.147 (NS)

* *means significant difference, p-value<0.05*
NS means no significant difference.

In table (4), the subjects were in stage II, which is the beginning of the puberty. There was a significant difference in the age between males and females. Also there was a significant difference in the body weight, indicated that males gained weight more than females in this stage. There were no significant differences between both sexes regarding the other parameters. So in the first and second stages of sexual development, BMD did not differ significantly between males and females.

Table (5): Comparison of parameters between males and females in pubertal stage III

Parameters	Male (n=21) mean±SD	Female (n=17) mean±SD	p-value
Age (year)	13.96±1.3	11.9±1.3	<0.001**
Weight (Kg)	51±8.19	42.29±10.7	<0.05*
Height (cm)	158.95±6.8	148.65±6.76	<0.001**
BMI (Kg/m^2)	20.2±2.9	19±3.4	0.245 (NS)
BMD (g/cm^2)	0.702±0.05	0.658±0.04	<0.05*
Calcium (mmol/l)	2.38±0.1	2.39±0.08	0.71 (NS)
Phosphorus (mmol/l)	1.33±0.32	1.7±0.54	<0.05*
ALP (IU/l)	187±59.9	200.18±62.5	0.514 (NS)

* *means significant difference, p-value<0.05*

** *means highly significant difference, p-value<0.001*

NS means no significant difference.

In table (5), the subjects were in stage III, the gender differences were noticed in some parameters (age, body weight, height, BMD and phosphorus). So, females were progressing in puberty earlier than males, while the males showed continued gains in mean values of body weight in addition to height and BMD more than those of females. When compared with the results for males at this stage, the mean phosphorus level for females was higher than that for males. However there were no significant differences in BMI, calcium and ALP.

Table (6): Comparison of parameters between males and females in pubertal stage IV

Parameters	Male (n=13) mean±SD	Female (n=18) mean±SD	p-value
Age (year)	15.9±0.9	13.7±1.12	<0.001 **
Weight (Kg)	57.64±13	45.43±7.7	<0.05 *
Height (cm)	164.85±6.49	152.11±6.12	<0.001 **
BMI (Kg/m^2)	21.14±4.2	19.6±2.7	0.221 (NS)
BMD (g/cm^2)	0.801±0.06	0.776±0.06	0.234 (NS)
Calcium (mmol/l)	2.37±0.1	2.38±0.1	0.825 (NS)
Phosphorus (mmol/l)	1.09±0.25	1.3±0.3	<0.05*
ALP (IU/l)	117.38±31.5	155.94±68.78	0.07 (NS)

* *means significant difference, p-value<0.05*

** *means highly significant difference, p-value<0.001*

NS means no significant difference.

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In table (6), the subjects were in stage IV, the gender differences as in stage III, were noticed in age, body weight, height and phosphorus, but disappeared in BMD. Regarding body weight as in stages II and III, males had more mean values than females. As in stage III, phosphorus level was still higher in females than in males with no significant differences in BMI, calcium and ALP.

Table (7): Comparison of parameters between males and females in pubertal stage V

Parameters	Male (n=25) mean±SD	Female (n=21) mean±SD	p-value
Age (year)	17.7±1.4	17±1.1.8	0.169 (NS)
Weight (Kg)	66.6±11.2	52.68±8.13	<0.001 **
Height (cm)	168.56±65.14	156.62±5.45	<0.001 **
BMI (Kg/m^2)	23.3±3	21.4±2.6	<0.05 *
BMD (g/cm^2)	0.927±0.11	0.928±0.07	0.975 (NS)
Calcium (mmol/l)	2.38±0.1	2.4±0.1	0.328 (NS)
Phosphorus (mmol/l)	1.05±0.2	1.1±0.19	0.507 (NS)
ALP (IU/l)	81.28±27.64	90.38±50.3	0.441 (NS)

* *means significant difference, p-value<0.05*

** *means highly significant difference, p-value<0.001*

NS means no significant difference.

In table (7), the subjects were in stage V (the stage of adulthood). Significant differences were found in the anthropometric measurement (body weight, height and BMI) between males and females; males had significantly greater mean values in these measurements, but there were no significant differences in other parameters (age, BMD, calcium, phosphorus, and ALP).

The effect of puberty on the parameters:

The effect of puberty on the outcomes was assessed by using one-way analysis of variance (ANOVA) followed by Duncan´s multiple range tests on each gender separately (table-8 for males, table-9 for females). The mean values were presented by letters (a, b, c, d, e); from a to e, the values were increasing. The means that have different letters horizontally indicate significant differences at p-value<0.05.

Table (8): Comparison of parameters between different pubertal stages in male subjects

Parameters	Stage I	Stage II	Stage III	Stage IV	Stage V
Age (year)	10.98 (a)	12.66 (b)	13.96 (c)	15.89 (d)	17.7 (e)
Weight (Kg)	33.61 (a)	43.6 (b)	51.02 (c)	57.64 (d)	66.6 (e)
Height (cm)	142.39 (a)	150 (b)	158.95 (c)	164.85 (d)	168.56 (e)
BMI (Kg/m^2)	16.53 (a)	19.32 (b)	20.2 (b)	21.14 (b)	23.34 (c)
BMD (g/cm^2)	0.518 (a)	0.598 (b)	0.702 (c)	0.801 (d)	0.927 (e)
Calcium (mmol/l)	2.32 (a)	2.32 (a)	2.38 (a)	2.37 (a)	2.38 (a)
Phosphorus (mmol/l)	2 (c)	1.68 (b)	1.33 (a)	1.09 (a)	1.05 (a)
ALP (IU/l)	228.72 (d)	250.21 (d)	187 (c)	117.38 (b)	81.28 (a)
Testosterone (ng/ml)	0.1 (a)	0.86 (a)	4.68 (b)	6.73 (c)	8.02 (d)

The means that have different letters horizontally indicate significant differences at p-value<0.05

There was an increase in all parameters between the five pubertal stages (except for calcium, phosphorus and ALP). These increases exhibiting significant differences across all pubertal stages. The main

increase in height occurred between stages II and III, and the main increase in BMD between stages IV and V. So subjects in either Tanner stage IV or V had higher values for BMD than those at earlier stages of sexual development.

Calcium seemed unchanged among different stages. Phosphorus and ALP levels varied significantly with pubertal stages; being higher at early puberty (I and II) and declined in mid and late puberty (III, IV and V). A significant increase in testosterone levels were observed between all Tanner stages except in the early stages (I and II).

Table (9): Comparison of parameters between different pubertal stages in female subjects

Parameters	Stage I	Stage II	Stage III	Stage IV	Stage V
Age (year)	10.4 (a)	11.48 (b)	11.9 (b)	13.66 (c)	17.05 (d)
Weight (Kg)	32.67 (a)	37.18 (a,b)	42.29 (b,c)	45.43 (c)	52.68 (d)
Height (cm)	140.11 (a)	146 (b)	148.65 (b,c)	152.11 (c)	156.62 (d)
BMI (Kg/m^2)	16.53 (a)	17.37 (a,b)	19 (b,c)	19.6 (c,d)	21.4 (d)
BMD (g/cm^2)	0.512 (a)	0.583 (b)	0.658 (c)	0.776 (d)	0.928 (e)
Calcium (mmol/l)	2.36 (a)	2.34 (a)	2.39 (a)	2.38 (a)	2.41 (a)
Phosphorus (mmol/l)	2.03 (c)	1.98 (c)	1.71 (b)	1.31 (a)	1.09 (a)
ALP (IU/l)	186.78 (b,c)	235.9 (d)	203.3 (c,d)	150.6 (b)	90.38 (a)
E_2 (pg/ml)	9 (a)	9 (a)	32.97 (a)	146.5 (b)	234.3 (c)

The means that have different letters horizontally indicate significant differences at p-value<0.05.

As for males, females showed a significant increase in BMD that parallel the increase in age, body weight, height, and BMI. Calcium also remained within the same range in all stages, with decreasing in phosphorus and ALP levels as progressing in the pubertal stages.

The main increase in height occurred between stages I and II, and the main increase in BMD occurred between stages IV and V. So subjects in either Tanner stage IV or V had higher values for BMD than those at earlier stages of sexual development. E_2 mean levels in stage I and II were the same because the detection limit of the minividas is <9 pg/ml; E_2 increased slightly in stage III but not significantly, whereas a pronounced increase was detected in E_2 levels at stages IV and V.

Gender-specific values of BMD by age group

The BMD values were described according to age groups for each gender separately. They were subdivided into 8 age groups at one-year intervals in each. In this study, there were no significant differences in BMD between age 17 and more than 17 years (the upper limit of this study was 20.2 years), so the age of 17 years and more was considered as one group.

Table (10) shows a comparison of BMD between males and females in each age group by using unpaired *t*-test, while the effect of age on BMD values by a comparison of BMD values between different age groups in males and females was assessed by using one-way analysis of variance (ANOVA) followed by Duncan´s multiple range tests on each gender separately.

The mean values were presented by letters (a, b, c, d, e); from a to e, the values were increasing. The means that have different letters vertically indicate significant differences at p-value<0.05.

Table (10): Comparison of BMD between males and females in each age group

Age groups(year)	male		Female		p-value
	No. of subjects	BMD g/cm²	No. of subjects	BMD g/cm²	
10-10.9	13	0.536 (a)	16	0.598 (a)	<0.05 *
11-11.9	6	0.595 (b)	13	0.618 (b)	0.771(NS)
12-12.9	8	0.613 (b)	12	0.658 (b,c)	0.123(NS)
13-13.9	18	0.638 (b)	11	0.756 (c,d)	<0.05 *
14-14.9	7	0.675 (b)	5	0.796 (d)	<0.05 *
15-15.9	12	0.770 (c)	8	0.808 (d)	0.688(NS)
16-16.9	11	0.806 (c)	11	0.915 (e)	<0.05 *
17 and >	21	0.924 (d)	5	0.920 (e)	0.837(NS)

* *means significant difference, p-value<0.05*
NS means no significant difference.
The means that have different letters vertically indicate significant differences at p-value<0.05.

Comparison between males and females in each age group was done by using unpaired *t*-test. There were no significant differences between males and females until the age of 10. After that, females presented higher values for BMD than males in all other age groups, probably because of the earlier onset of puberty in females, and the significant differences were at the age of 10-10.9, 13-13.9, 14-14.9 and at 16-16.9 years. By the age of 17 years and more, the values for males were greater than those for females, but the difference was non significant.

BMD increased significantly with age, the increase occurred especially after age of 15 years. There was other period of acceleration in lumbar spine BMD, between age groups 16-17 years in males. Whereas the lowest variation was present between age groups 11-12 years.

For female subjects, regular increase in BMD values in the different age groups was found, the increase was significant every 2 years interval. The lowest variations were present in age group 10-11 and 16-17 years, while the maximal increase occurred around age of 13 years in females (approximately two years later in males, around age of 15). There were other periods of acceleration in lumbar spine BMD at the beginning of puberty (after age 10 years) and between age groups 15-16 years.

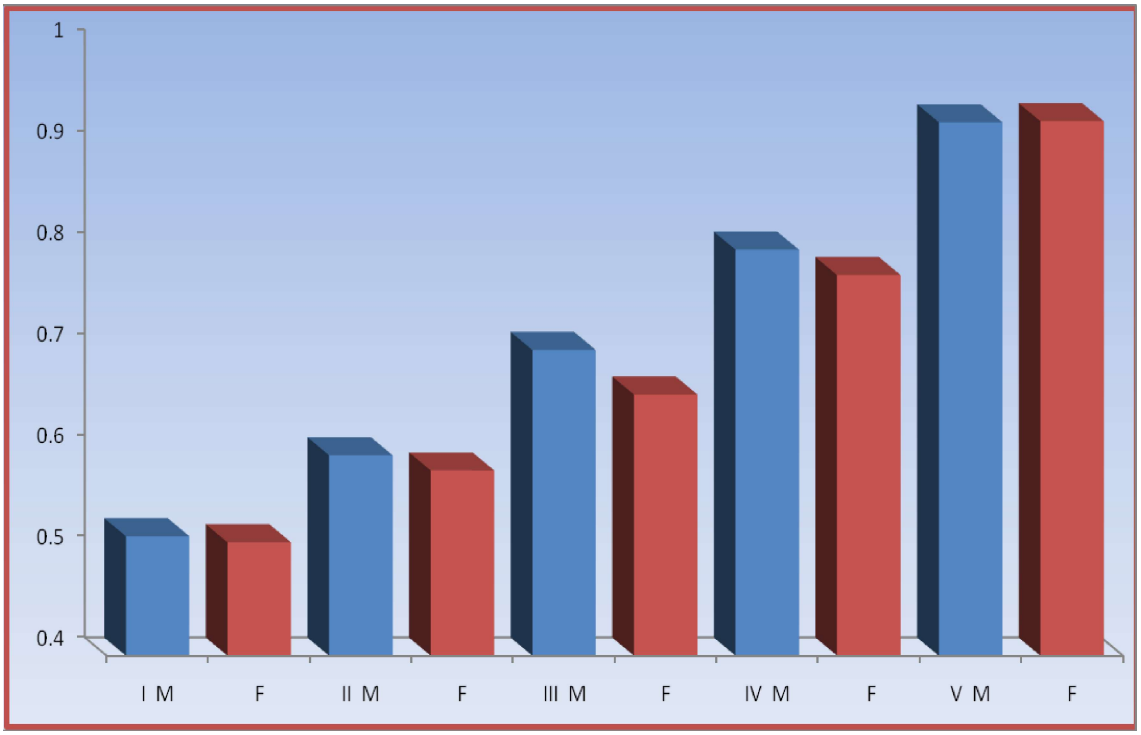

Figure (13): Comparison of BMD between males and females in each pubertal stage (M=male, F=female).

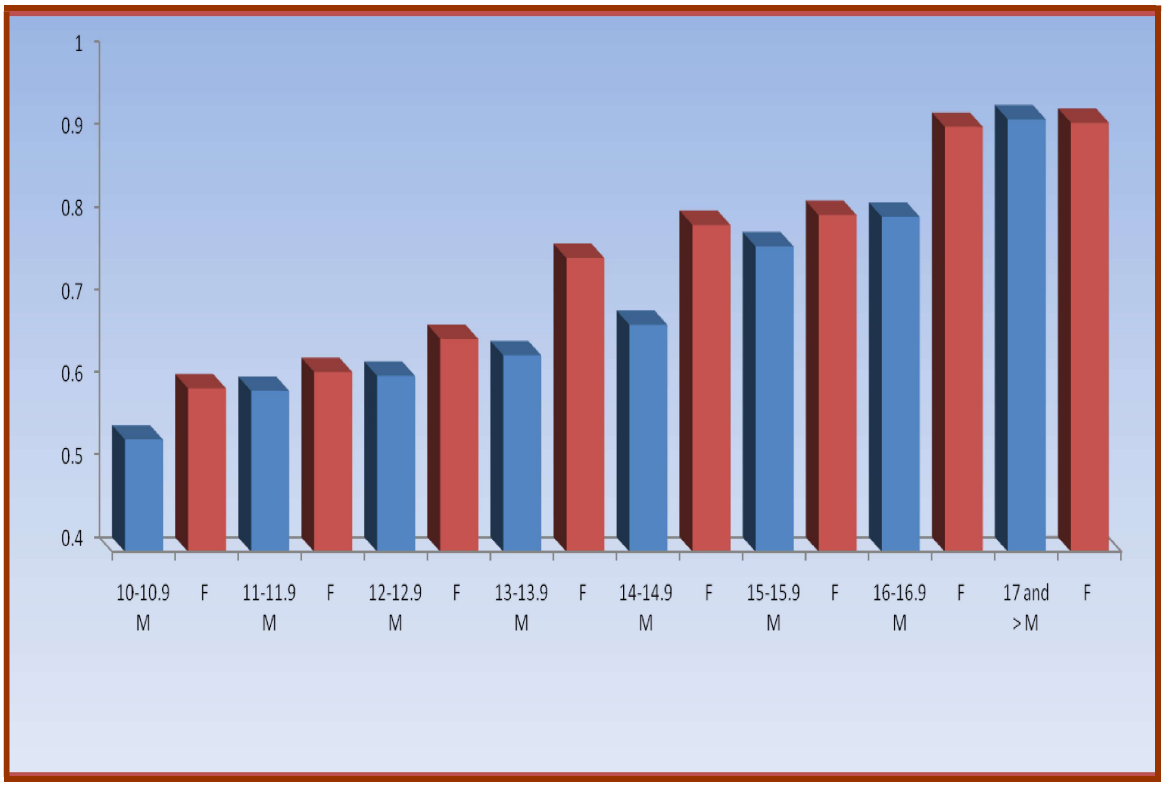

Figure (14): Comparison of BMD between males and females in each age group (M=male, F=female).

Correlations between BMD and other parameters: Pearson's correlation coefficient was used to find the relationships between BMD and the other parameters, these correlations were represented as scatter diagrams for each parameter alone and for both sexes separately.

Correlation between BMD and age:

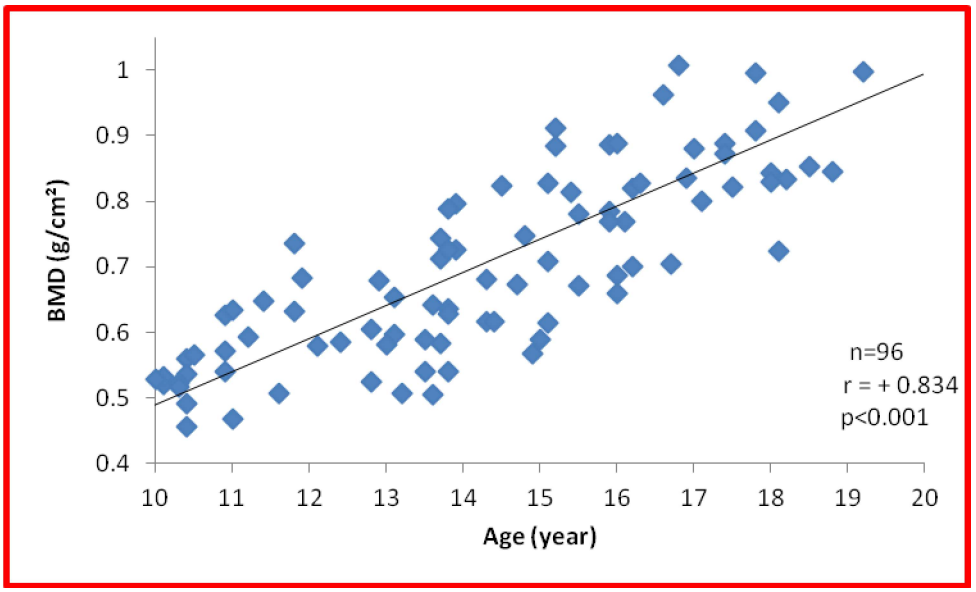

Figure (15): Scatter diagram representing the correlation between BMD and age in males.

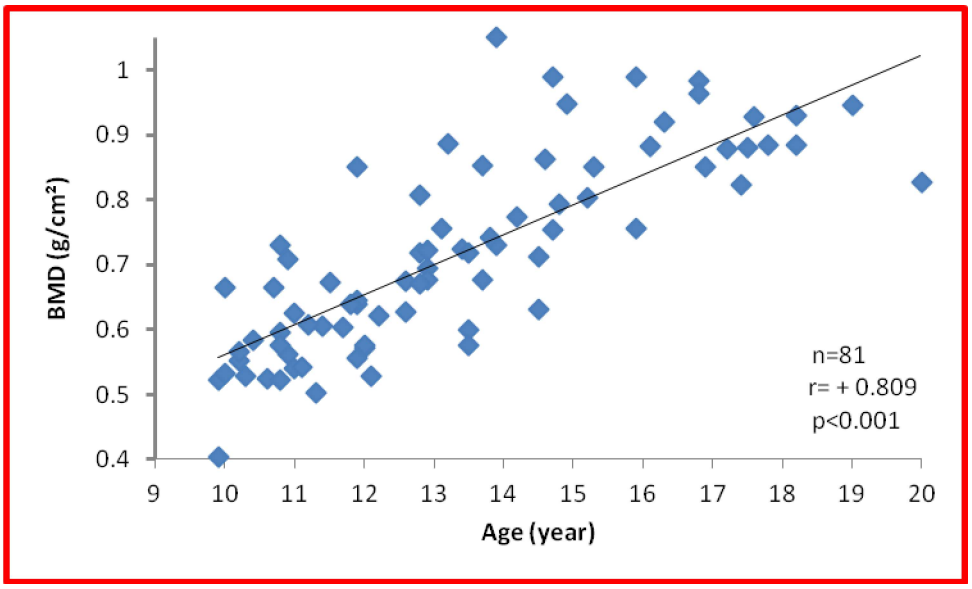

Figure (16): Scatter diagram representing the correlation between BMD and age in females.

Correlation between BMD and body weight:

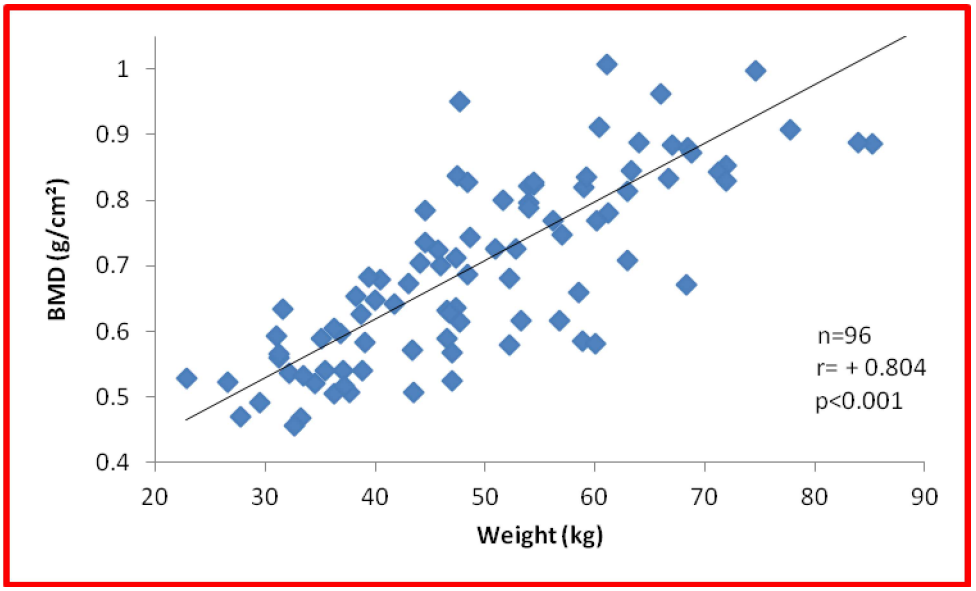

Figure (17): Scatter diagram representing the correlation between BMD and body weight in males.

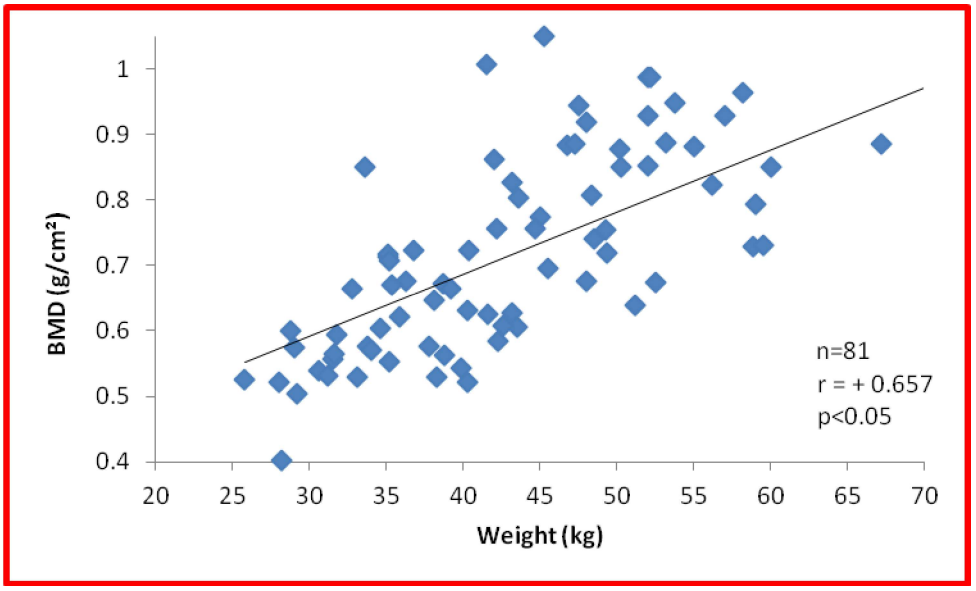

Figure (18): Scatter diagram representing the correlation between BMD and body weight in females.

Correlation between BMD and height:

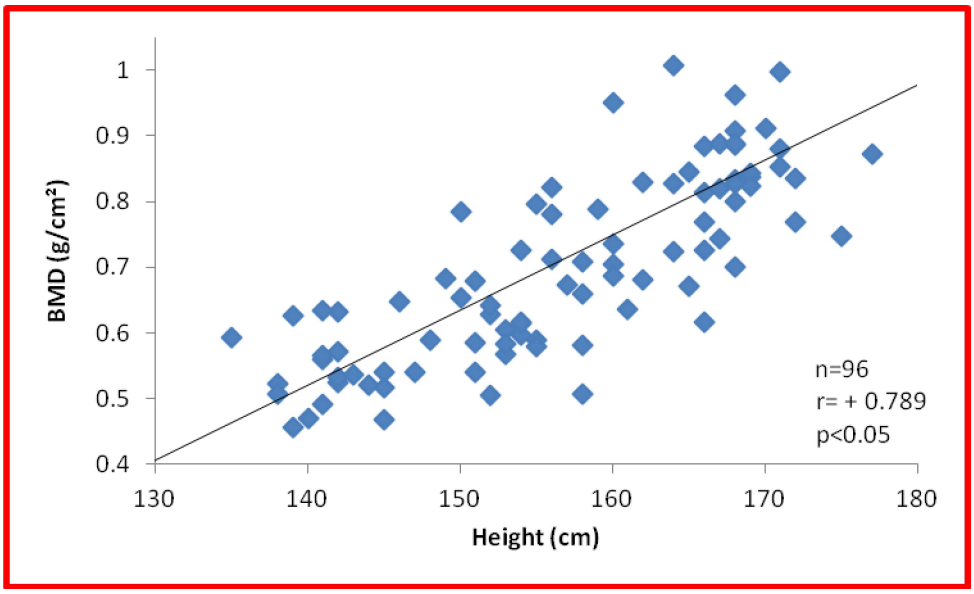

Figure (19): Scatter diagram representing the correlation between BMD and height in males.

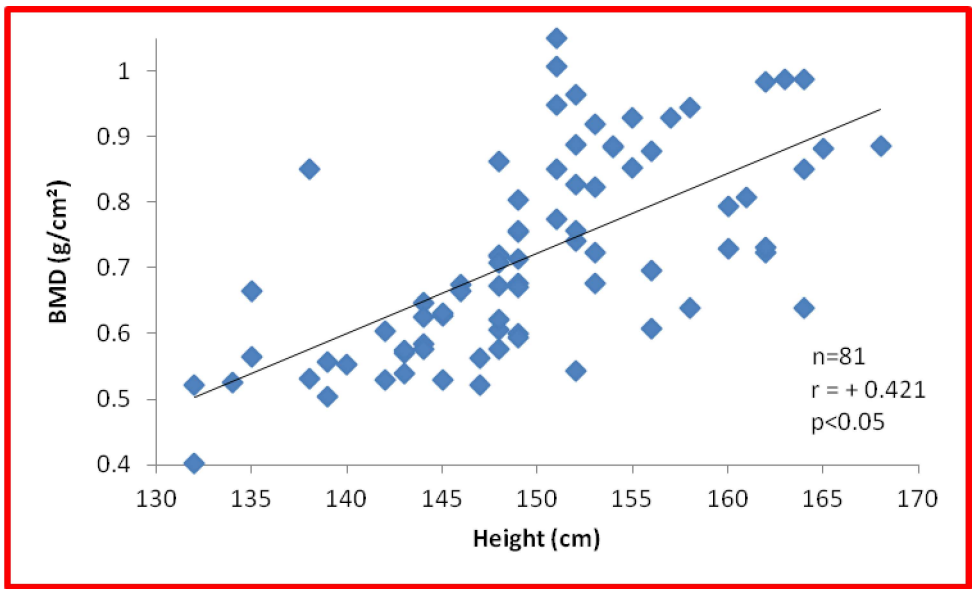

Figure (20): Scatter diagram representing the correlation between BMD and height in females.

Correlation between BMD and BMI:

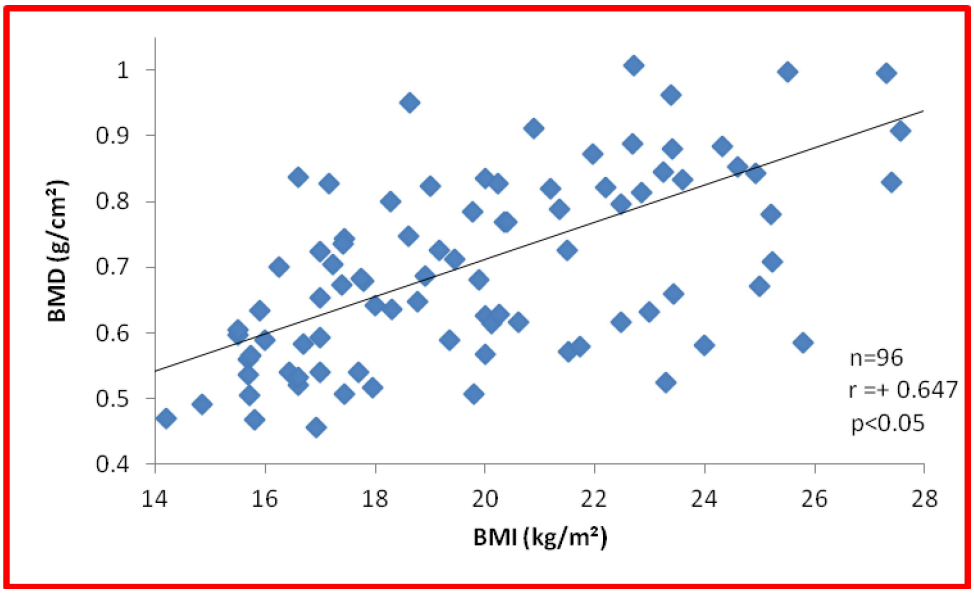

Figure (21): Scatter diagram representing the correlation between BMD and BMI in males.

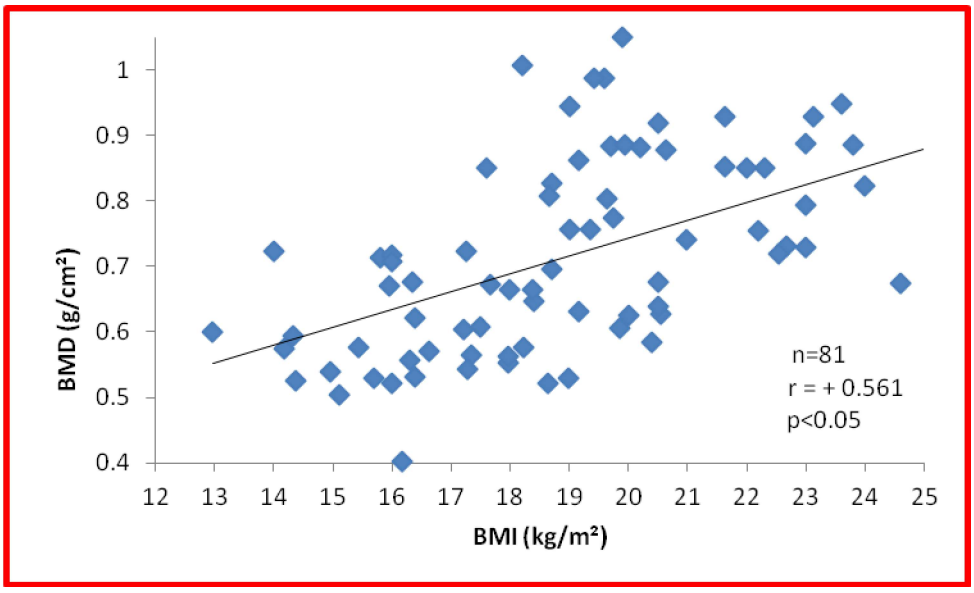

Figure (22): Scatter diagram representing the correlation between BMD and BMI in females.

Correlation between BMD and serum calcium:

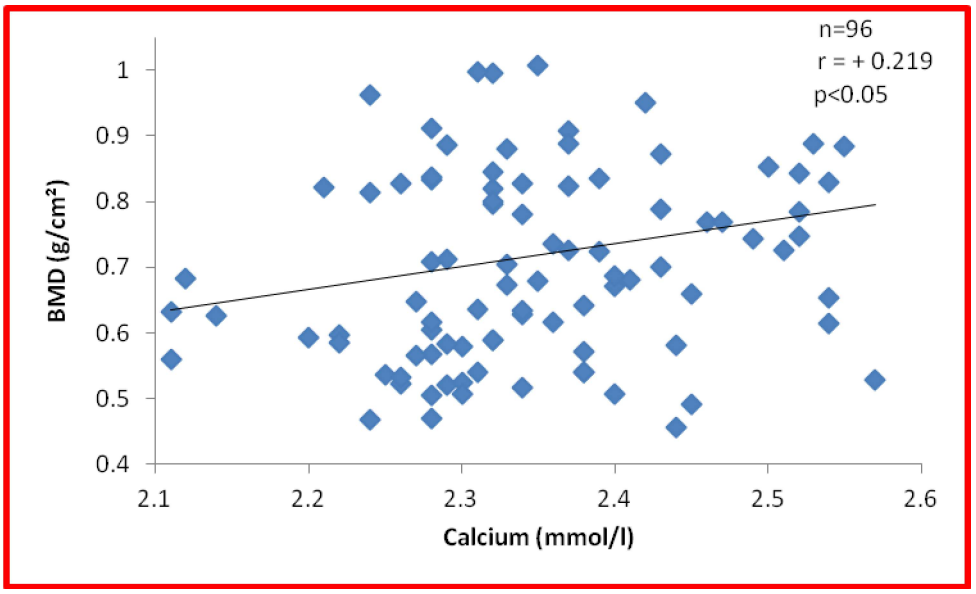

Figure (23): Scatter diagram representing the correlation between BMD and serum calcium in males.

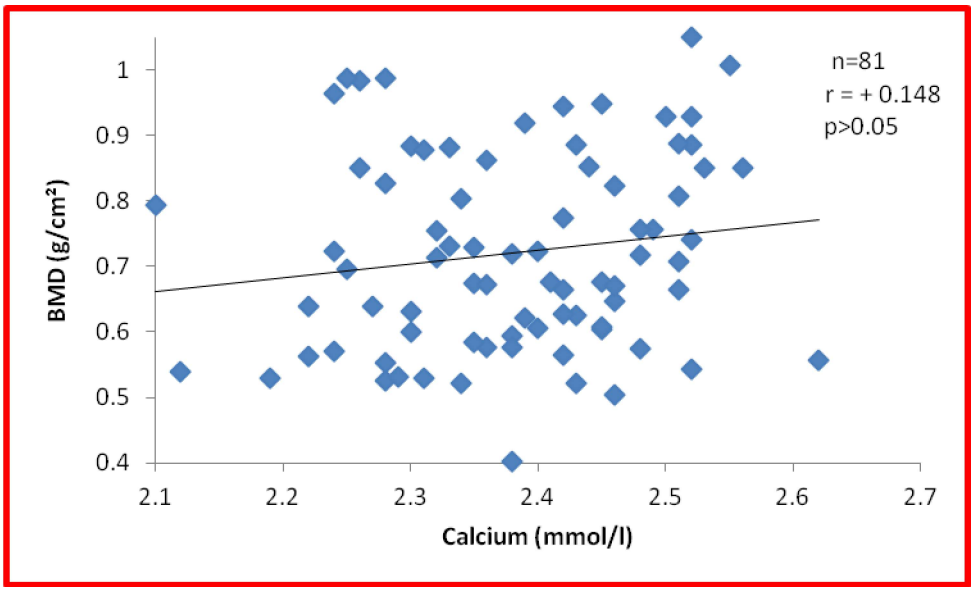

Figure (24): Scatter diagram representing the correlation between BMD and serum calcium in females.

Correlation between BMD and serum phosphorus:

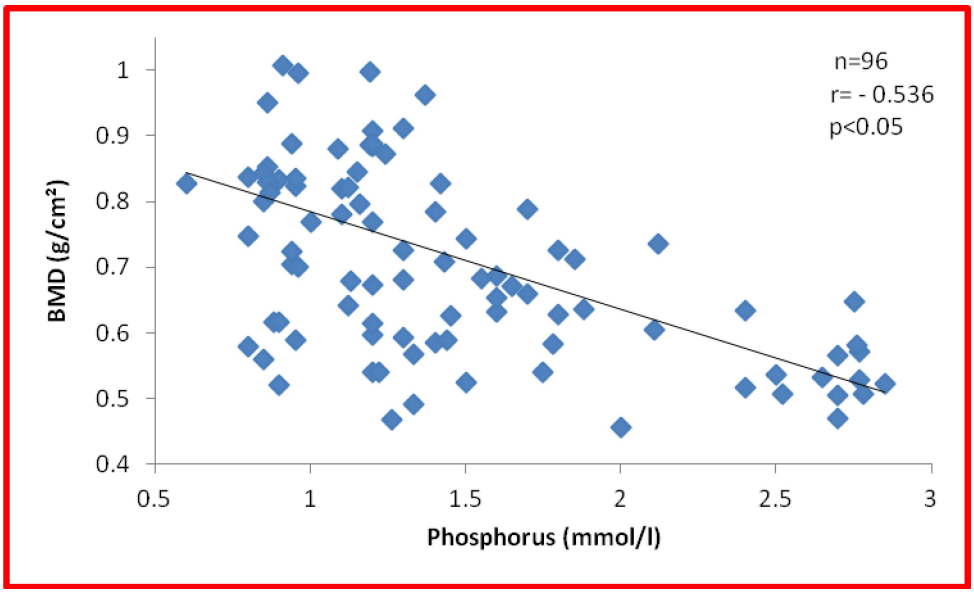

Figure (25): Scatter diagram representing the correlation between BMD and serum phosphorus in males.

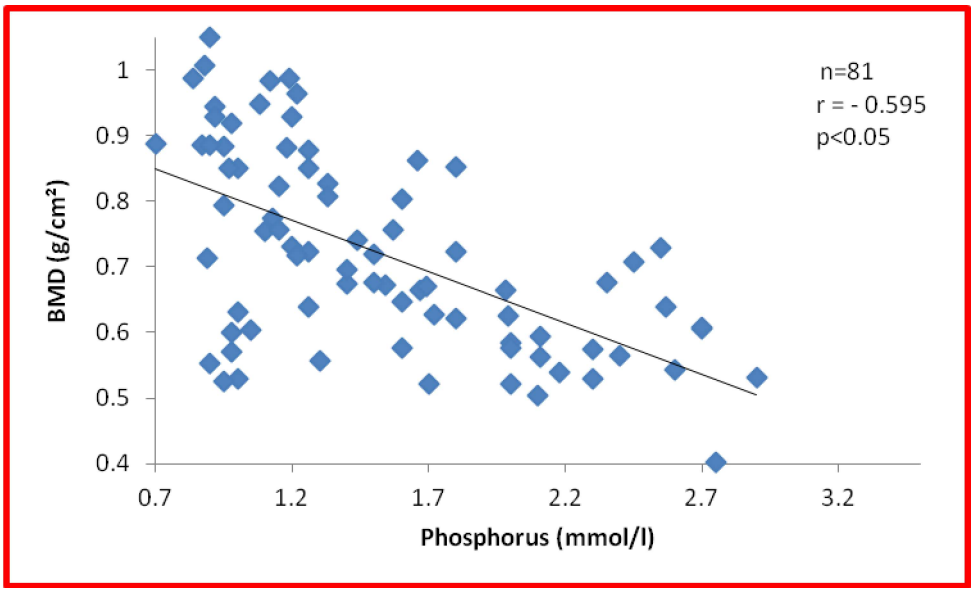

Figure (26): Scatter diagram representing the correlation between BMD and serum phosphorus in females.

Correlation between BMD and serum ALP activity:

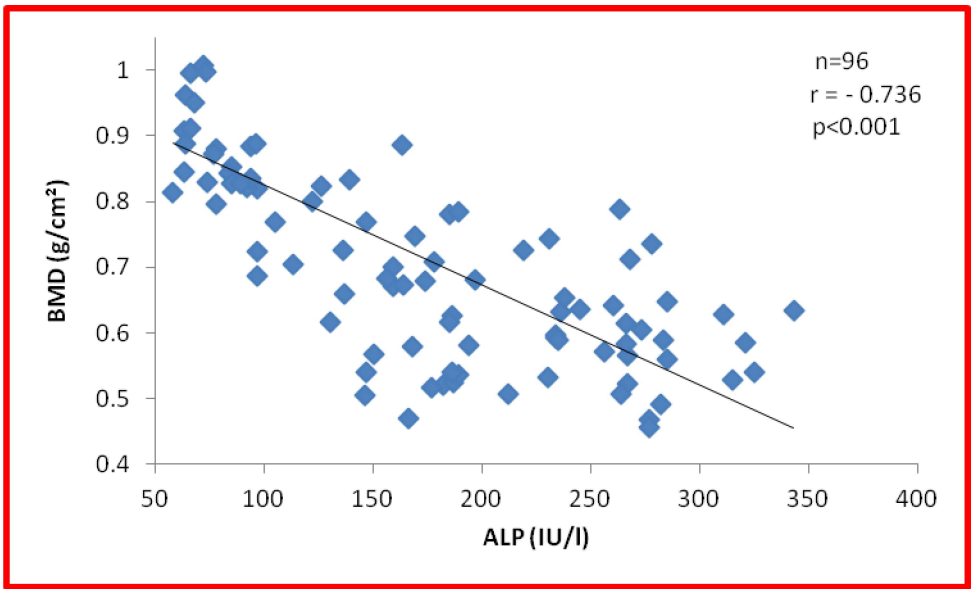

Figure (27): Scatter diagram representing the correlation between BMD and serum ALP activity in males.

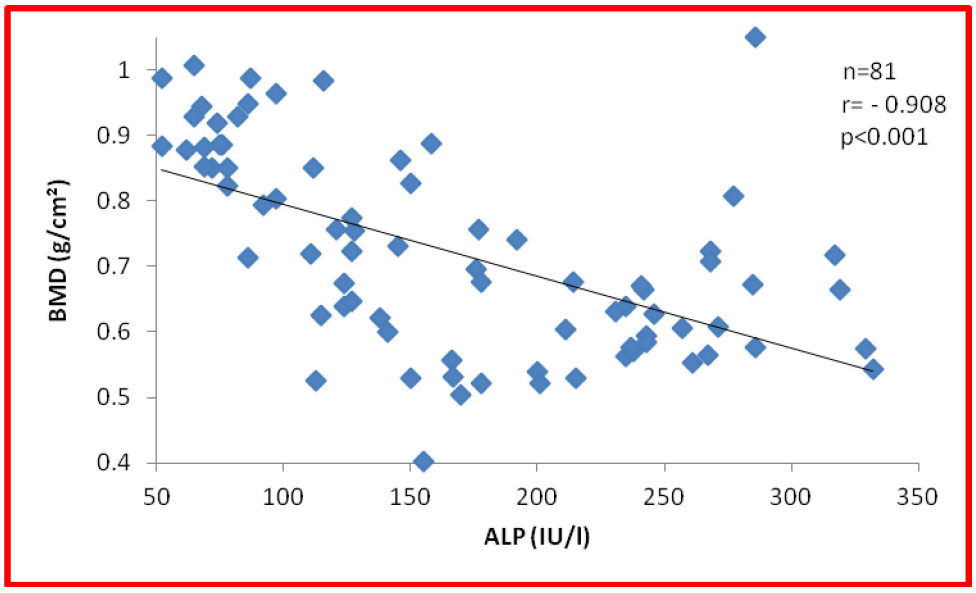

Figure (28): Scatter diagram representing the correlation between BMD and serum ALP activity in females.

Correlation between BMD and sex steroids:

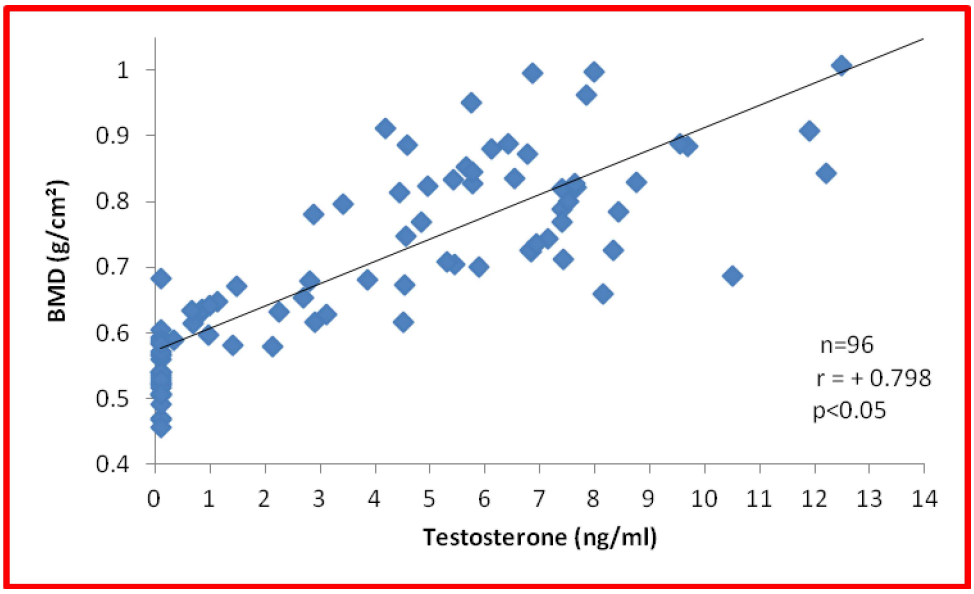

Figure (29): Scatter diagram representing the correlation between BMD and testosterone in males.

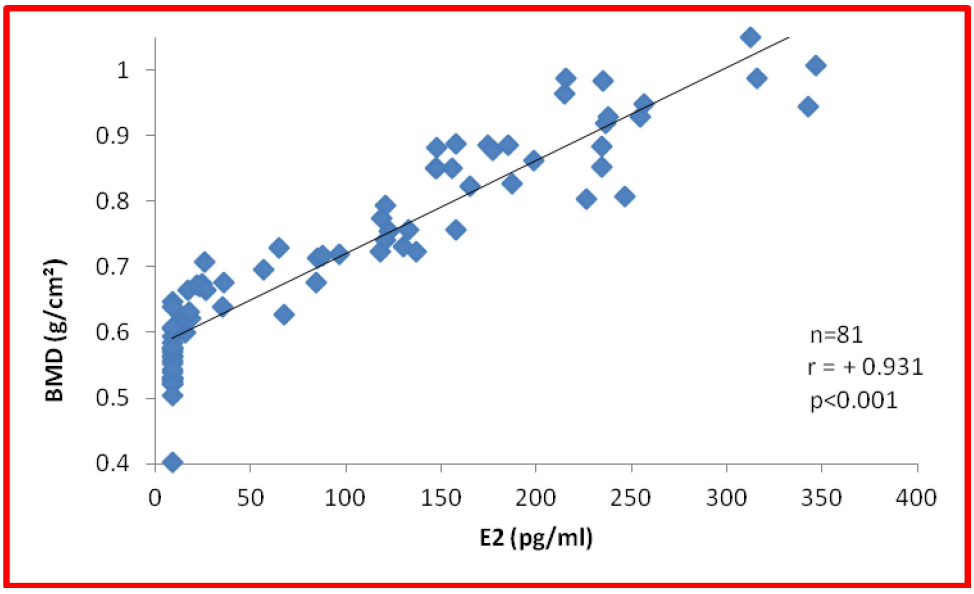

Figure (30): Scatter diagram representing the correlation between BMD and E2 in females.

Correlation between sex steroids and ALP activity:

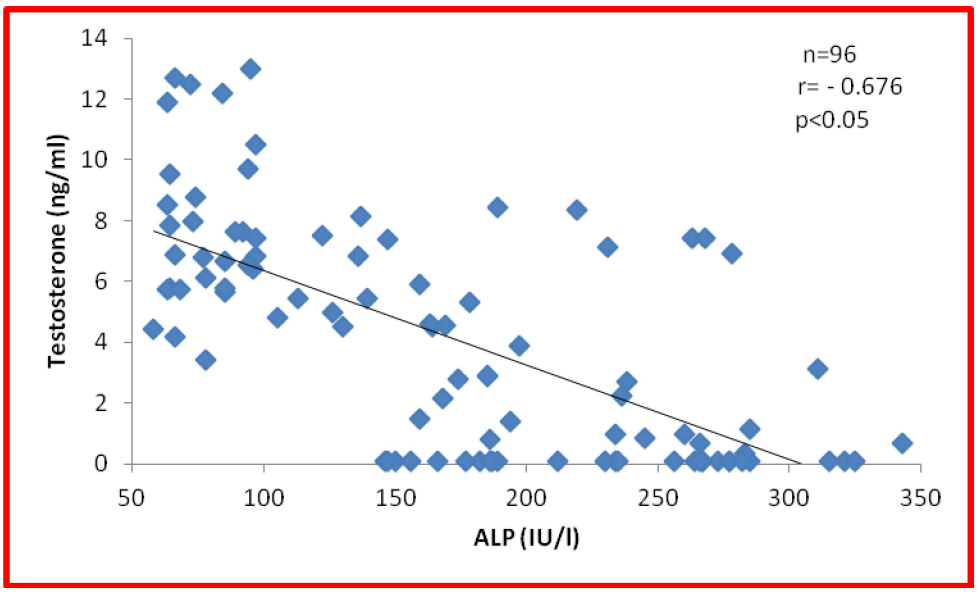

Figure (31): Scatter diagram representing the correlation between testosterone and ALP activity in males.

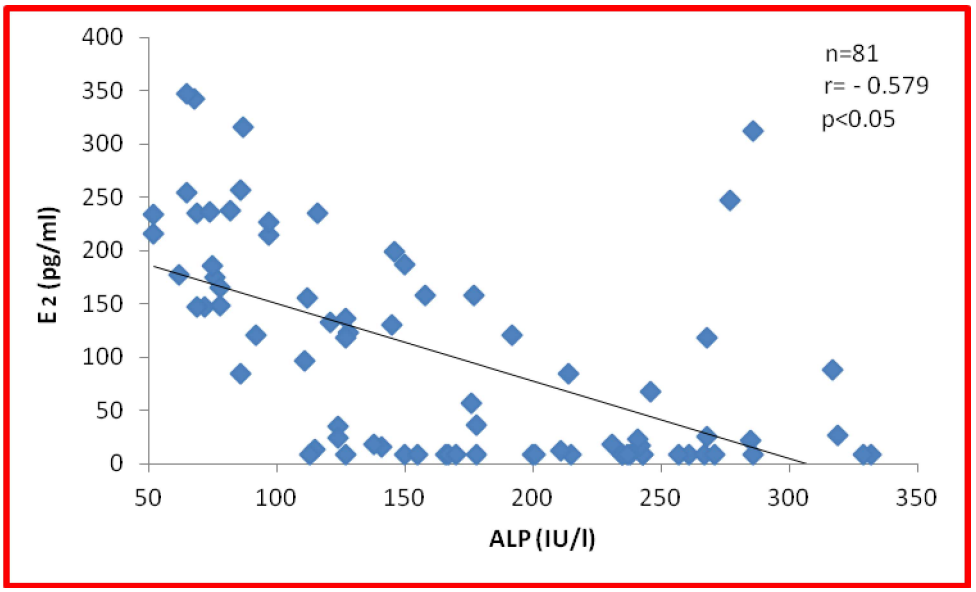

Figure (32): Scatter diagram representing the correlation between E2 and ALP activity in females.

Association of various parameters with BMD

Stepwise multiple regression analysis was performed in order to evaluate the impact of the combination of several parameters on BMD values. Age, body weight, height, BMI, pubertal stages, calcium, phosphorus, ALP, and testosterone for male and E2 for female, were entered into each regression model as continuous independent predictors, and BMD as dependent variable.

Table (11): Association of various parameters with BMD

Parameters	Male		Female	
	p-value	Standardized coefficients Beta	p-value	Standardized coefficients Beta
Weight (Kg)	<0.001**	0.544		
Height (cm)	<0.05*	0.551		
BMI (Kg/m^2)	<0.05*	0.687		
Pubertal stages	<0.001**	0.578	<0.001**	0.453
Testosterone (ng/ml)	<0.05 *	0.186		
E2 (pg/ml)			<0.001**	0.541

* *means significant difference, p-value<0.05*

** *means highly significant difference, p-value<0.001*

Beta coefficients showed the direction of the relationship between variables, If it is positive, then the relationship of this variable with dependent variable is positive and the vise is versa.

The factors pubertal stage, testosterone, body weight, height and BMI had significant influence on lumber spine BMD of males (R^2=91%). Whereas, pubertal stage and E2 were the factors with significant influence on lumber spine BMD of females (R^2=88%). So it can be concluded that

pubertal stage was the major predictor for BMD values in both sexes. Testosterone and E2 levels were the factors with significant influence on BMD of males and females, respectively.

In addition to that, body weight, height and BMI were the factors with significant influence on BMD for males only but not for females.

Ethnic differences:

The mean of total lumber spine Z-scores was calculated, this Z-scores in the study group were derived through the densitometer software using an American database as reference.

For females, the mean Z- scores was: -1.2±1.2. For males, the mean Z-scores was: -1.4±1.1. Comparing these Z-scores demonstrated that the mean values of BMD in healthy subjects from Mosul city were lower than those of age- and gender-matched American children.

Discussion

The present study provided gender-specific lumber spine BMD values, expressed in discrete age and pubertal stage subgroups, and were measured in pubertal males and females aged between 10-20.2 years. Furthermore it described pubertal increments in BMD at the lumber spine concomitant with the significant increase in body dimensions and its relationship with maturation of secondary sexual characteristics.

Effect of anthropometric parameters on BMD:

In the current study, there were significant increases of the anthropometric parameters (body weight, height, and BMI) across all pubertal stages for both sexes. In prepubertal stage, there were no significant differences between males and females in these parameters. After this stage, significant gender differences in weight and height began to appear in stage II and stage III, respectively. Whereas BMI did not show such gender differences till stage V. As anticipated, males were significantly taller, heavier and with greater BMI than females.

A delay between the maximum gain in height and maximum gain in BMD for both sexes has been noticed. The main increase in height for the male subjects occurred between stages II and III, while for females between stages I and II, and both sexes showed the main increase in BMD between stages IV and V, which suggesting a clear relationship between the increase of sex steroids, linear growth and bone growth.

These findings more closely correspond to those obtained by Theintz *et al*.,1992 and Fournier *et al*.,1997, who observed that during pre-pubertal period, gains in BMD and height follow a similar pattern, although during puberty it is no longer synchronous and they observed a lag period between PHV and PBV, that is, PHV precedes PBV.

In the present study, positive significant correlations between BMD and anthropometric parameters were demonstrated in both sexes (figures 15-20). On the other hand, such parameters were regarded as predictors for males BMD only and not for females BMD. These differences between males and females in the prediction of anthropometric parameters for BMD, could be explained by the larger size of male skeleton, due to the effects of androgen which speed up the radial growth of bones and increase bone dimensions by higher periosteal bone formation.

These results were similar to what was previously reported by Kröger *et al*.,1993 and Lu *et al*.,1994, who found that body weight and height correlated with BMD in both sexes.

Other studies observed the dependence of BMD on growth parameters, but in different patterns: Harel *et al*.,2007, for example, found a positive correlation between BMD and BMI in females. Boot *et al*.,1997; Fonsecal *et al*.,2001 and Silva *et al*.,2007, correlated BMD with anthropometric variables in males only. Goksen *et al*.,2006, found the correlation of BMD with height only in both sexes, while Afghani *et al*.,2003; Faulkner *et al*.,1996 and Zhang *et al*.,2004, found that weight was one of the important factors influencing BMD in both sexes.

Effect of age on BMD:

For males as well as females, a progressive increase in BMD values in the different age groups was found in the present study and the correlation between age and BMD was significant for both sexes (figures 13 and 14). This is in agreement with the findings of Lu *et al.*,1996; Bachrach *et al.*,1999; Khan *et al.*,2004 and Kalkwarf *et al.*,2007. On similar ground, Sluis *et al.*,2002, suggested that the increase of lumbar spine BMD with age does reflect a real increase in mineralization, and is not merely a result of accelerated growth.

In all age groups, an important variation in the velocity of mineral gain was noticed in the current study. After an initial period with slight increases in BMD values, a period of rapid growth and accumulation of BMD in the lumbar spine was observed, especially striking around 13 and 15 years of age in females and males, respectively. There were another periods of acceleration in BMD, between age groups 15-16 years in females and 16-17 years in males. On the other hand, the lowest variation of BMD accrual was present between the age groups 11-12 years in males, and 10-11 and 16-17 years in females.

The acceleration of BMD gain in females, which occurred later in males, seems to be associated with pubertal growth, as females enter puberty earlier than males.

Bonjour *et al.*,1991; Boot *et al.*,1997; De Andrade *et al.*,2010 and Pérez-López *et al.*,2010, observed that the increase in BMD in females was rapid between 11-15 years of age, compared with 14–17 years in males, though the rate of change in BMD slowed dramatically by the age of 16-18 years in females and 17-20 years in males. Bailey *et al.*,1999 and Lim,2010, reported that the main increase in BMD was between the ages 11–13 in females and 13–15 in males. `While Sluis *et al.*,2002, showed a

major acceleration in lumbar spine BMD from 13 years in males, which stabilized around age 15 to 16 years.

In the present study, age was not a predictor for BMD, this because age is limited in relation to the sequence of the events of puberty due to wide range of chronological ages within each pubertal stage. Furthermore, the increase in BMD was not steady, with periods of distinct rates of bone mineralization being observed. This finding was in agreement with that mentioned by Gilsanz *et al*., 1991 and Fonseca *et al*.,2001.

Effect of puberty on BMD:

In this study, BMD was compared with degree of sexual maturity, in an attempt to identify those pubertal stages that indicated the greatest increment in BMD. A significant increase in BMD values was observed among all pubertal stages, and occurred earlier in females than in males.

Similarly, Glastre *et al*.,1990; Rio del *et al*.,1994 and Nguyen *et al*.,2001, observed a significant increase in BMD values according to puberty. Moreover, De Schepper *et al*.,1991 and Silva *et al*.,2007 observed a linear increase in BMD during childhood, with an exponential increase during puberty.

In the present study, both sexes showed the main increase in BMD between stages IV and V, during which the deceleration of the growth spurt occurs and adult levels of sex steroids can be attained. These high sex steroids levels at stage IV and V was thought to enhance BMD accrual. Males showed higher BMD values than females according to pubertal stage subgroups, but without significant differences between them in all stages except in stage III.

This is in accordance with what was previously reported by De Schepper *et al*.,1991 and Hasanoğlu *et al*.,2004.

Silva *et al*.,2004 and 2007, obtained the same findings in males; in that BMD values increase significantly across all pubertal stages and the main increase was in stage IV and V, with the exception that there were no significant alterations in BMD between stage I and stage III.

Contrary to the findings in this study, Slemenda *et al*.,1994 and Gracia-Marco *et al*.,2010, showed that the most important increase in BMD takes place between Tanner stages III and IV in both sexes. While, Bonjour *et al*.,1991 and Libanati *et al*.,1999, noticed a rapid accumulation of BMD between pubertal stages II and IV.

The present study confirmed the powerful effect of puberty on BMD, a significant positive correlation between them has been found and the pubertal stage has been regarded as the main determinant of BMD values in both sexes. So pubertal staging should be considered when interpreting BMD measurements with DXA.

In agreement with these results, Martin *et al*.,1997, observed that pubertal development was a significant predictor of BMD. While, Boot *et al*.,1997, found that the influence of puberty on BMD was higher in females than in males, and the major determinant of BMD during puberty appeared to be weight in males and pubertal development in females. Cheng *et al*.,1998 and Arabi *et al*.,2004, showed that weight, along with age and pubertal stages, should be considered in an estimate of BMD in pubertal children. Seeman *et al*.,1989 and Gilsanz *et al*.,1991, stated that the rate of bone accretion increases dramatically during puberty and is a function of pubertal stage, rather than chronological age.

Adult BMD values were found in the present study in subjects with stage V pubertal development. However, this does not mean that these

BMD values represent the PBM, which is generally attained around the age of 30 years (Pérez-López *et al*.,2010).

Gender differences in BMD:

In the current study, a comparison between the both sexes was done according to age subgroups and pubertal stage subgroups. However, some gender differences in the accrual of BMD were evident.

When gender differences have been compared depending on age subgroups (figure-12), females had greater BMD at lumber spine across all age groups, as a result of the earlier onset of the puberty. Significant differences were seen at the age of 10-10.9, 13-14.9 and 16-16.9 years, whereas in late adolescence (around the age of 17, when most adolescents have completed their pubertal development) BMD values in males and females showed no significant differences.

Consistent with these data, Arabi *et al*.,2004; and Yilmaz *et al*.,2005, showed that females were shown to have greater vertebral BMD than males between age 10-16 years. While, Bonjour *et al*.,1991 and Faulkner *et al*.,1996, reported significant sex differences at ages 12-13 years.

Bailey *et al*.,1999, in a longitudinal study, observed that the main BMD accrual occurs approximately 1.6 year earlier in females than in males (age 12.5±0.90 and 14.1±0.95 years, respectively). McCormick *et al*.,1999 and Pérez-López *et al*.,2010, likewise, found that in females BMD accrual rates reach the maximum around age 11-14 while in males there is a 2-3 years delay due to pubertal gender differences.

In the present study, when gender differences have been compared depending on pubertal stages (figure-10), There were no significant differences between them except in stage III, in that males had higher

BMD values than females, this indicated that males gain more BMD than females at this stage, which may be due to the significant increase of testosterone levels in males at this stage, whereas E_2 did not increase significantly at stage III in females. The absence of gender differences in prepubertal years (stage I) indicated that the development of BMD before puberty is not dependent on sex steroids.

This coincides with the results of Maynard *et al.*,1998 and Hasanoğlu *et al.*,2004, who observed that gender differences in BMD values at all pubertal stage were non significant. In addition to that, Rio del *et al.*,1994 and Nguyen *et al.*,2001, did not observe gender differences in prepubertal years.

In contrast, Moro *et al.*,1996 and Martin *et al.*,1997, reported that males displayed significant higher BMD as compared to females in all stages of puberty, and the greater male BMD has been postulated to be due to the specific effects of androgens, although it has also been related to increases in mechanical loading.

In view of these results, one may conclude that the gender differences in BMD may be explained by many factors, such as body size, muscle mass, and the difference in the level of physical activity**.** In addition to that**,** the pattern of bone growth in males differs from that in females in 2 ways: First, males have 2 more years of prepubertal growth because of the later onset of puberty (age 13, rather than 11 as in females). Second, their pubertal growth spurt lasts for 4 years rather than the 3 years in females (Riggs *et al.*,2002; Meier and Kraenzlin,2007). Studies on animals suggested the existence of sex-linked genes mediating the gender difference in BMD (Orwoll *et al.*,2001).

The relationship between sex steroids and BMD:

In the present study, there was increase in levels of serum testosterone and E2 with progressing pubertal stages in males and females, respectively, until adult levels were achieved at the end of puberty, this increase was accompanied by an increment in BMD values.

The low testosterone levels in early puberty (stage I and II) may be involved in promoting linear skeletal growth much more than skeletal mineralization. After that, in stage III, there was a surge in testosterone, and this high testosterone level at mid and late puberty in males was thought to enhance BMD accrual as well as linear skeletal growth via direct or indirect stimulation of bone formation. This finding was similar to that reported by Riis *et al*.,1985 and Klein *et al*.,1996.

In the present study, E2 level in females began to increase during stage III, although it was non significant, and then E2 increased dramatically thereafter during stage IV and V. This increase in E2 levels during puberty may be involved in promoting both linear growth and skeletal mineralization in females, also, E2 has important effects on BMD through modulating bone remodeling by suppression of bone turnover at the endosteal surface leading to an increase in cortical and trabecular thickness.

Significant positive correlations were found between sex steroids and BMD in the present study (figures-27 and 28). Furthermore, the impact of sex steroids on BMD was evaluated by using stepwise regression analysis, and E2 was regarded as a positive predictor for BMD in females and testosterone in males.

The above results were in consistent with findings of Slootweg 1993; Van Coeverden *et al*.,2002 and Wang *et al*.,2006, while Yilmaz *et al*.,2005 found a modest correlation between BMD and testosterone in males. Wang *et al*.,2004 found that lumber spine BMD was positively

correlated with E2 levels even in prepubertal and in early pubertal females.

On the other hand, the correlations between testosterone and E2 with ALP in males and females, respectively, were significant negative correlations (figures-29 and 30). From these results, it can be concluded that in late puberty, the high levels of sex steroids inhibit chondrocyte proliferation, which leads to decline in height velocity and an immediate decline in bone turnover markers, whereas BMD continues increasing under the influence of sex steroids. This suggests that sex steroids may be responsible for the reduction of bone turnover in late puberty.

Similar to the above results, Cadogan *et al*.,1998 and Yilmaz *et al*.,2005, found a significant correlation between E2 levels and ALP in females, but non significant one for testosterone and ALP in males.

Menarcheal age and BMD:

To evaluate whether the age at menarche influenced BMD in the present study, a correlation between BMD values and menarcheal age was performed to 44 females who had experienced menarche, there was no significant correlation between BMD and menarcheal age for these females (p-value=0.241). This may be explained by the fact that most of the females in the study had menarcheal age within narrow limit; the mean age at menarche was 13.1 ± 1.2 years, that there was neither too early nor too late menarche.

These findings conflict with the conclusions of Boot *et al*.,1997; Afghani *et al*.,2003 and Chevalley *et al*.2009, who found that BMD was lower in the later than the earlier menarcheal group.

Changes in serum calcium during puberty and its relation to BMD:

The mean calcium concentration for the subjects enrolled in this study was (2.36±0.1 for males and 2.38±0.1 for females), which was within the normal range (Mclntosh *et al*.,2008). The serum concentrations of calcium remained remarkably constant throughout puberty, and unchanged from normal adult values. No significant differences were noticed between both sexes at any pubertal stage. This probably reflects the important functions of calcium ion which is regulated by complex interplay among the skeleton, intestine, kidneys, and parathyearoid gland. The findings obtained in this study were equivalent to those obtained in other studies (Round *et al*.,1973 and Pettifor *et al*., 1978).

In the present study, the correlation of calcium with BMD was significant in males (figure-21), while it was non significant in females (figure-22). In addition to that, serum calcium level was not a predictor for BMD in both sexes.

Although there is increase in calcium gain by the skeleton, children are in positive bone balance (formation > resorption), which ensures healthy skeletal growth to achieve the maximum PBM. A positive calcium balance is due to changes in calcium metabolism, with increase in intestinal absorption of calcium as an early phenomenon during puberty (Peacock,2010). This may be due to the role of sex steroids which stimulate calcium absorption and retention and result in a net positive flow of calcium into bone (Žofkova,2008 and Mejia *et al*.,2009).

Furthermore, some authors suggested that this calcium absorptive efficiency during puberty may be genetically regulated (Abrams *et al*.,2005). This is analogous to the increase in calcium absorption during pregnancy that occurs in the second trimester before the maximum

utilization of calcium by the fetus in the third trimester (Abrams *et al*.,2000; Ott,2008).

Changes in serum inorganic phosphorus during puberty and its relation to BMD:

The mean phosphorus level for the subjects enrolled in this study was (1.42±0.6 for males and 1.55±0.6 for females) which was within the normal range (Mclntosh *et al*.,2008). Phosphorus levels varied significantly with pubertal stages being higher at early puberty (stage I and II) and declined in mid and late puberty (stage III, IV and V), then decrease toward adult values in both genders. There was significant difference between males and females in stage III and IV being higher in females. The correlation between serum phosphorus level and BMD was significant negative one (figures- 23 and 24), though serum phosphorus level was not a predictor for BMD in both sexes. These data strongly support the assumption that the known changes of phosphorus levels are mainly due to growth and probably secondarily to sex hormone changes. These changes in phosphorus concentration are readily tolerated and the physiologic range is wide, that children have much higher levels than adults, in addition to that, phosphorus absorption is rarely limited.

The above results are in agreement with Round *et al*.,1973 and Widhalm and Hölzl,1985, who showed that serum phosphorus levels remain high in childhood, increase slightly with acceleration of growth and pubertal development. they showed that males had significantly higher levels than females only at ages 13-15 years and 16-17 years.

Changes in serum ALP activity during puberty and its relation to BMD:

In the present study, ALP activity varied within a wide range (52-343 IU/l). The values of ALP activity in prepubertal children (stage I) were considered to be 3-4 times higher than in adults. The mean peak values of ALP were occurred at stage II in both genders, afterward, the activity decreased towards adult levels as the children become sexually mature. These marked changes of ALP levels during adolescence reflect that the bone metabolism rates are greater in children compared with adults.

These results were similar to those obtained in other studies (Van Coeverden *et al*.,2002 and Gracia-Marco *et al*.,2010).

Eapen *et al*.,2008 found that circulating bone markers are high in the first 2 years of life, decrease in childhood and increase again during puberty, so they regarded adolescence as a high bone-turnover state, with increased levels of both bone formation and resorption, then ALP levels decrease to adult levels in late puberty.

Similarly, Widhalm and Hölzl,1985; Libanati *et al*.,1999 and Mora *et al*.,1999, found that the peak values occur during early puberty (pubertal stage II) in both sexes. While Bennett *et al*.,1976 and Tobiume *et al*.,1997, demonstrated that ALP level was maximum in midpuberty (stage II and III), but decreased in late puberty. Fares *et al*.,2003, also observed that bone formation markers in females were highest in Tanner stages II and III and had decreased into the normal premenopausal ranges by stage V, conversely, in males, these markers were significantly elevated in early and midpuberty and remained above the normal range for normal young men, even by Tanner stage V.

Regarding gender difference in ALP levels, the current study revealed that there were no significant differences in all pubertal stages. In contrast to Gracia-Marco *et al.*,2010, who observed that males presented higher levels in all Tanner stages, while Fares *et al.*,2003, showed gender differences in bone formation markers, being higher in males at pubertal stages IV and V compared to females.

The results of this study showed that the correlation between ALP and BMD was significant negative one (figures- 25 and 26), but serum ALP activity was not a predictor for BMD in both sexes. It can be concluded that reduced rates of bone turnover play a major role in accretion of BMD during puberty. So ALP activity was correlated with height velocity and not with bone gain, as longitudinal growth is more rapid during early pubertal stages (when ALP levels are maximal during these stages), whereas bone mineral accrual is greater during mid and late pubertal stages, when ALP levels are declining, probably because of high levels of sex steroids and IGF-1.

This in agreement with Cadogan *et al.*,1998; Libanati *et al.*,1999 and Mora *et al.*,1999. Szulc *et al.*,2000, also suggested that bone turnover markers are likely to reflect statural growth rather than bone mineral accrual.

Eapen *et al.*,2008, also found that age related changes in serum ALP levels correlate positively and significantly with height velocity in both genders. Tuchman *et al.*,2008, mentioned that, in children and adolescents, biomarkers of bone metabolism represent the aggregate effects of endochondral bone formation, longitudinal growth, and increases in bone circumference and thickness, as well as bone remodeling.

Unlike the above findings, Van Coeverden *et al.*,2002, found a significant correlation between bone turnover markers and BMD in

males, but not in females, whereas Yilmaz *et al*.,2005, showed that bone formation markers were good predictors of bone mass in females, but not in males.

Ethnic differences in BMD:

Z-score is number of standard deviations higher or lower than the mean of peers matched for age, sex, and ethnicity. Z-scores in the study group were derived through the densitometer software using the American database as reference**.** It has been found that our pediatric and adolescent population have lower BMD values than Americans. The mean Z-scores were -1.2±1.2 for females and -1.4±1.1 for males. Nevertheless, such Z- scores were regarded within normal, because the term "low BMD for chronologic age" should be labeled when Z-score is less than or equal to -2 (Binkovitz *et al*.,2007). The differences of BMD values in the present study from Western values, may be explained by differences in the onset of puberty and in fact, there is no actual correction for pubertal stage in the DXA device used in this study, or may be explained, in part, by sedentary lifestyle and declining in physical activities of our children and adolescents.

There is an established ethnic difference in BMD (Bachrach *et al*.,1999; Kalkwarf *et al*.,2007), BMD in Asians is reported to be lower than in other nationalities (Venkat *et al*.,2009). Arabi *et al*.,2004, found that the mean BMD in healthy Lebanese pediatric subjects is lower than that of age- and gender-matched Canadian children.

On similar ground, Saadi *et al*.,2001, found a lower mean BMD for United Arab Emirates females than United States Caucasian and Chinese females. Pajouhi *et al*.,2004, examined 10-75 years-old Iranian healthy

women; their BMD was 3.9% higher than that of Japanese women, but it was 1.9%-2.8% lower than Belgian, English, and French women.

Little is known, however, about the factors that contribute to racial variations in BMD or the time of life when such differences become manifest. Racial differences could account for the discrepancy between races in the rate of increase in BMD during puberty, which is affected by numerous metabolic and hormonal changes occur during this period, including increases in the production of GH, gonadotropins, and sex steroid hormones (Gilsanz *et al*.,1991). Many researchers have observed that over the last century, the age of females at menarche in the United States and Europe has gradually decreased, that is, Western children enter puberty earlier than other nationalities, which is probably because of improved nutritional status (Boron and Boulpaep,2005). Other researchers explained ethnic differences by the differences in lifestyle or in anthropometric measurements (Finkelstein *et al*.,2002).

Discrepancies in the results among studies, that is, some of them are in agreement with the present study, while others are not, may be ascribed to a variety of factors. These include the fact that some studies did not take into account the pubertal stages, differences in the sample size, differences in methodology and using different techniques for analyzing the data. Moreover, differences between DXA manufacturers which might have not given identical results.

Conclusions

• BMD of the lumbar spine increased with age, although such increase was not steady, with periods of distinct rates of bone mineralization being observed. So, in the current study, age was not a predictor for BMD.

• For males, the main accumulation was observed in the age groups 15-17 years, while the lowest variation was present in the age group 11-12 years. Whereas for females, the maximal increase of BMD occurred around the age of 13 years and 15-16 years, while the lowest variation was present in the age group 10-11 and 16-17 years.

• BMD increased with progressing puberty (from early to late puberty), and the main increase occurred between stage IV and V in both genders.

• This study provided a strong evidence that pubertal development was consistent predictor of BMD in healthy children and adolescents.

• The increase in bone mineralization during puberty occurs at the same time as significant increases in body dimensions, so the correlations of anthropometric parameters with BMD were significant positive in both genders. While, they were predictors for BMD in males only and not in females.

• Females presented higher values for BMD than males in all age groups, probably because of the earlier onset of puberty in females. Significant differences were seen at the age of 10-10.9, 13-14.9 and 16-16.9 years, whereas in late adolescence (around the age of 17, when most adolescents

have completed their pubertal development) BMD values in males and females showed no significant differences.

- The gender difference in values of BMD by pubertal stage was non significant except in stage III, in that males had higher BMD values than females had.

- There was increase in levels of serum testosterone in males and E2 in females with progressing puberty, until adult levels were achieved at the end of puberty, and this increase was accompanied by an increment in BMD values. So testosterone and E2 levels were positive predictors for BMD in males and females, respectively.

- Serum calcium seemed unchanged among different pubertal stages and unchanged from normal adult values. It was not a predictor for BMD in both gender.

- Serum phosphorus and ALP levels decreased with progressing puberty in both genders, which reflect that the bone metabolism rates are greater in children compared with adults. Serum phosphorus and ALP activity were not predictors for BMD, but their correlations with BMD were significant negative in both gender

- Significant negative correlation between testosterone levels and ALP activity in males and between E2 levels and ALP in females.

- BMD values in the study group were lower than Western normative values, mean Z-score for girls was: -1.2±1.2 and for boys was: -1.4±1.1.

Recommendations

- Early bone health is a key to the achievement of high PBM in young adulthood and serves as the "bone bank" for the remainder of adult life, so the pediatricians' recognition of the factors which influence BMD in childhood is important. They can advise children and their families accordingly and influence public health policies which may lead to an improvement in children's diets and levels of habitual physical activity, thus reducing the risks of osteoporosis and fractures in later life.

- Densitometry data should be adjusted for pubertal status since both growth and puberty influence BMD accrual. Therefore, values adjusted for Tanner stages will be of particular significance in the evaluation of children and adolescence with pubertal or growth disorders.

- In fact it is very important to correlate bone acquisition in young subjects not only with chronological age or gender, but also with anthropometrical parameters.

- Larger population study is needed to establish reference database values for BMD in our city, enabling the calculation of specific Z-scores for children and adolescents in this region.

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Appendix (1)

Date of questionnaire:
Serial no:
Name: Sex:
Age: pubertal stage:
Ethnicity: Address:

Clinical measures:
Weight: Height:
BMI: BMD:

Past medical history:
History of chronic medications:
History of smoking: (+) or (-)
History of programmed sporting activities: (+) or (-)
Menstruation: (+) or (-) , regular menses: (+) or (-)
Age of menarche:
History of fracture:
Family history of osteoporosis: (+) or (-)

Lab. Analysis:

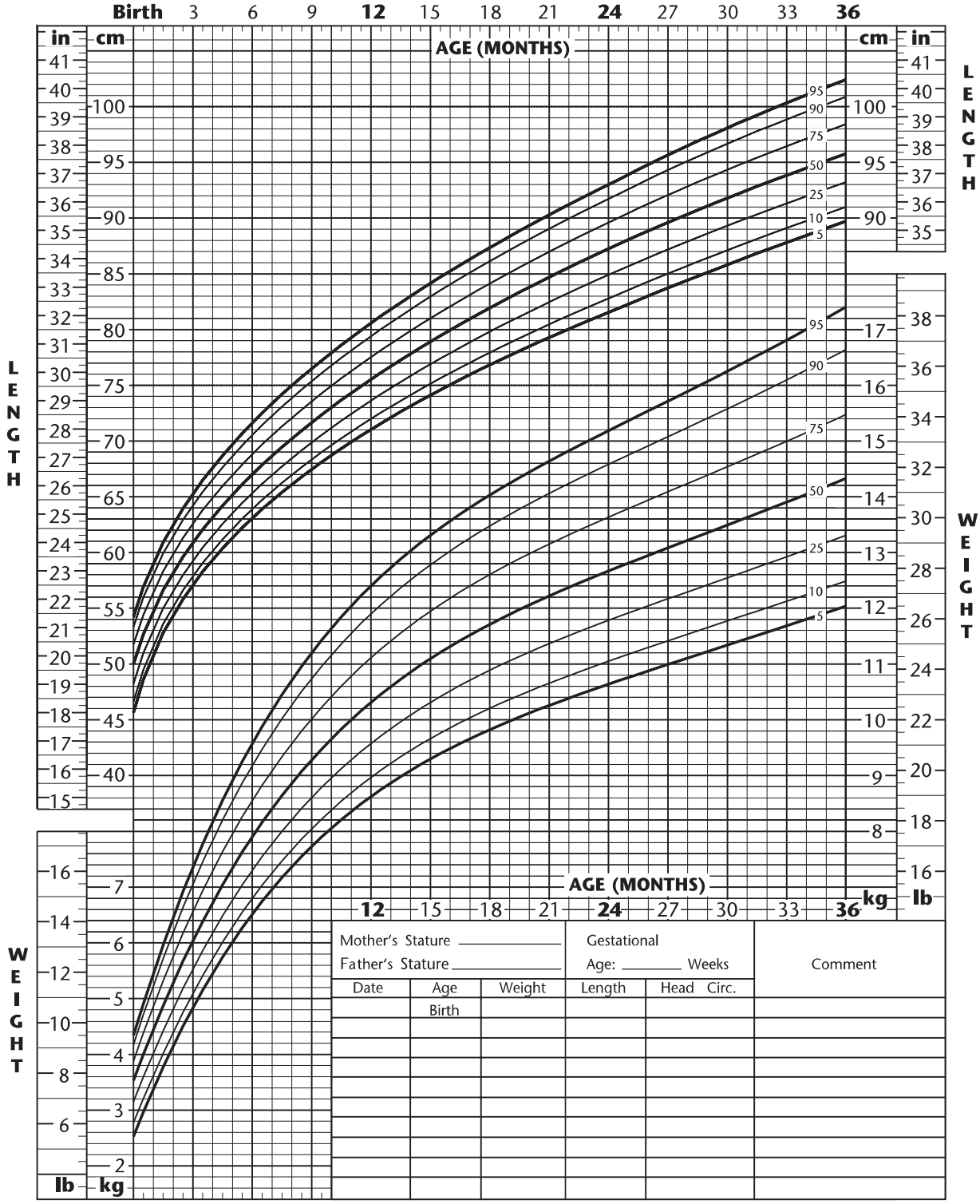
Serum calcium		mmol/l
Serum Albumin		(g/l)
Corrected calcium		mmol/l
Serum phosphorus		mmol/l
Serum total alkaline phosphatase		IU/l
Serum testosterone for male		ng/ml
Serum estradiol (E2) for female		pg/ml

Birth to 36 months: Boys

NAME _____

Length-for-age and Weight-for-age percentiles

RECORD # _____



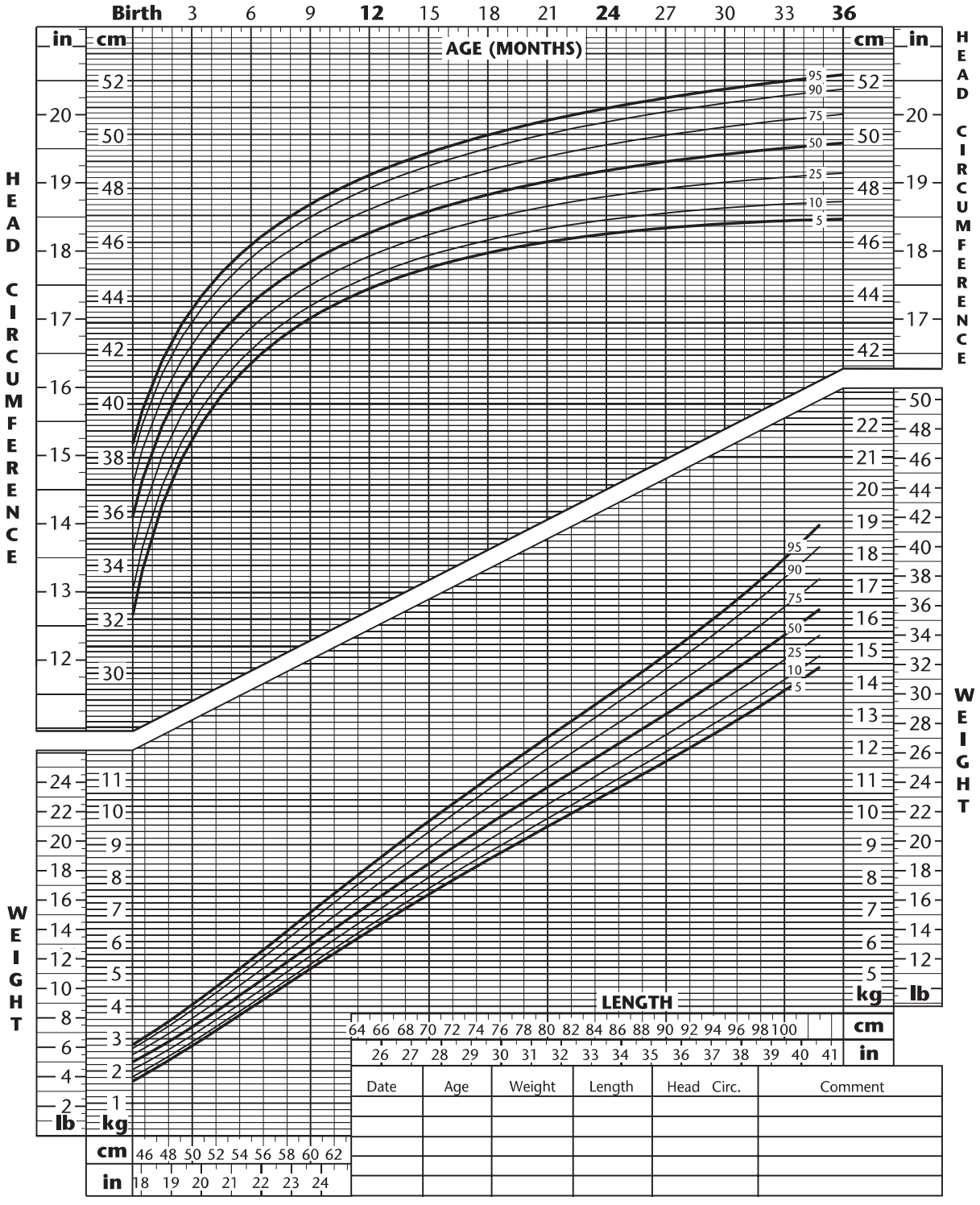
Revised April 20, 2001

SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000). **http://www.cdc.gov/growthcharts**

Birth to 36 months: Boys
Head circumference-for-age and
Weight-for-length percentiles

NAME _____

RECORD # _____



Date	Age	Weight	Length	Head Circ.	Comment

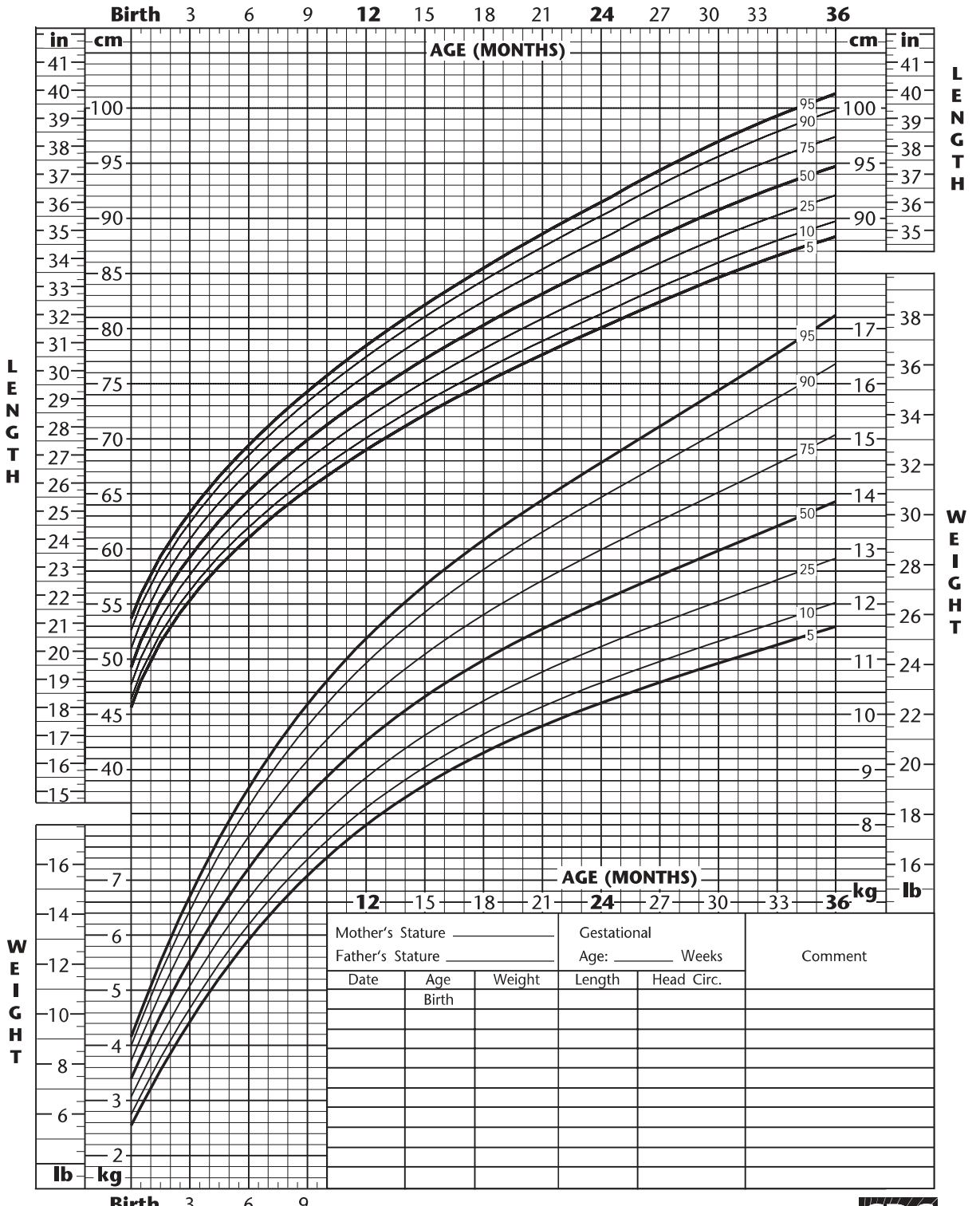
SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000). **http://www.cdc.gov/growthcharts**

Birth to 36 months: Girls

Length-for-age and Weight-for-age percentiles

NAME _____

RECORD # _____



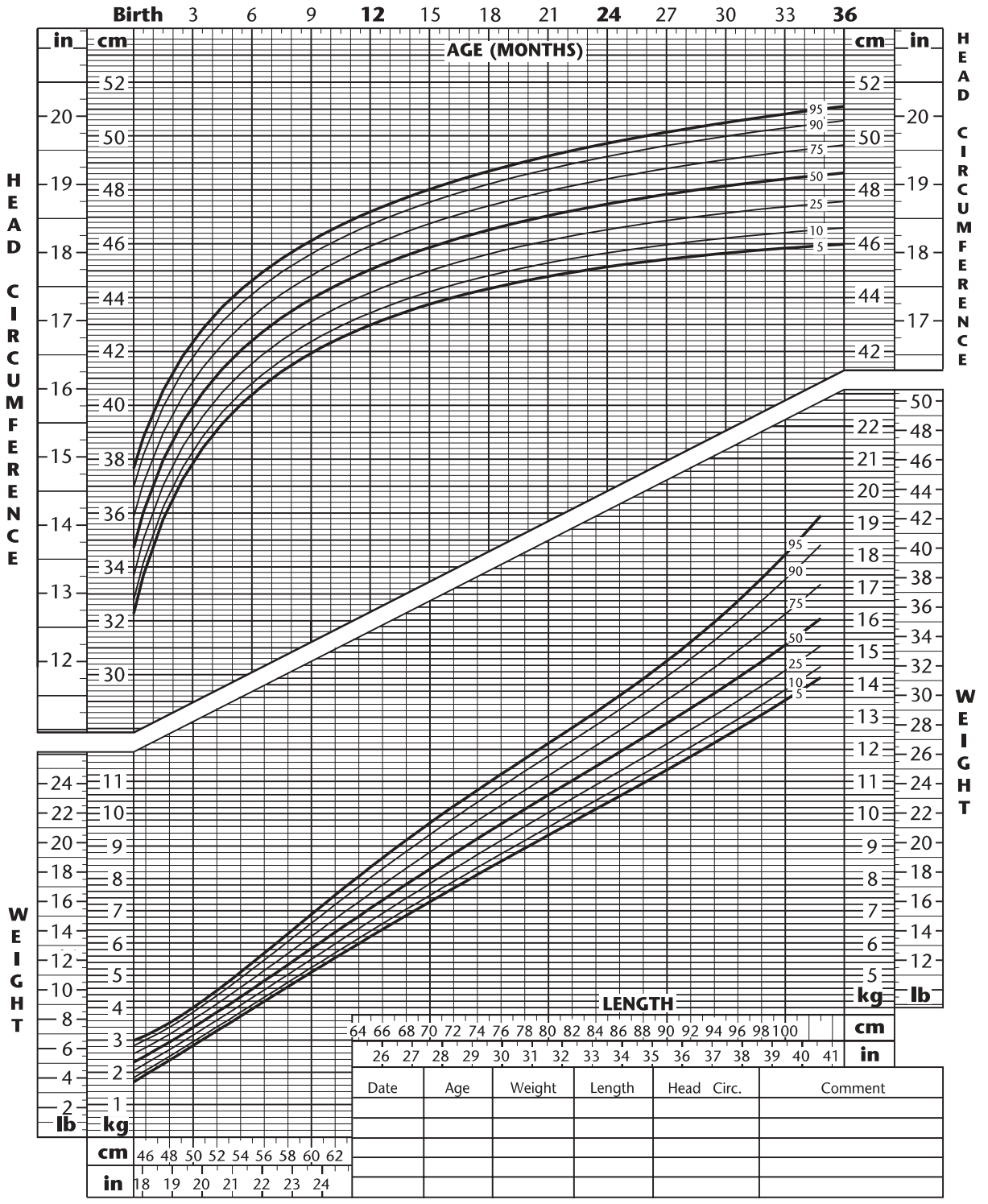
Revised April 20, 2001

SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000). **http://www.cdc.gov/growthcharts**

Birth to 36 months: Girls
Head circumference-for-age and
Weight-for-length percentiles

NAME _____

RECORD # _____



SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000). **http://www.cdc.gov/growthcharts**

2 to 20 years: Boys
Stature-for-age and Weight-for-age percentiles

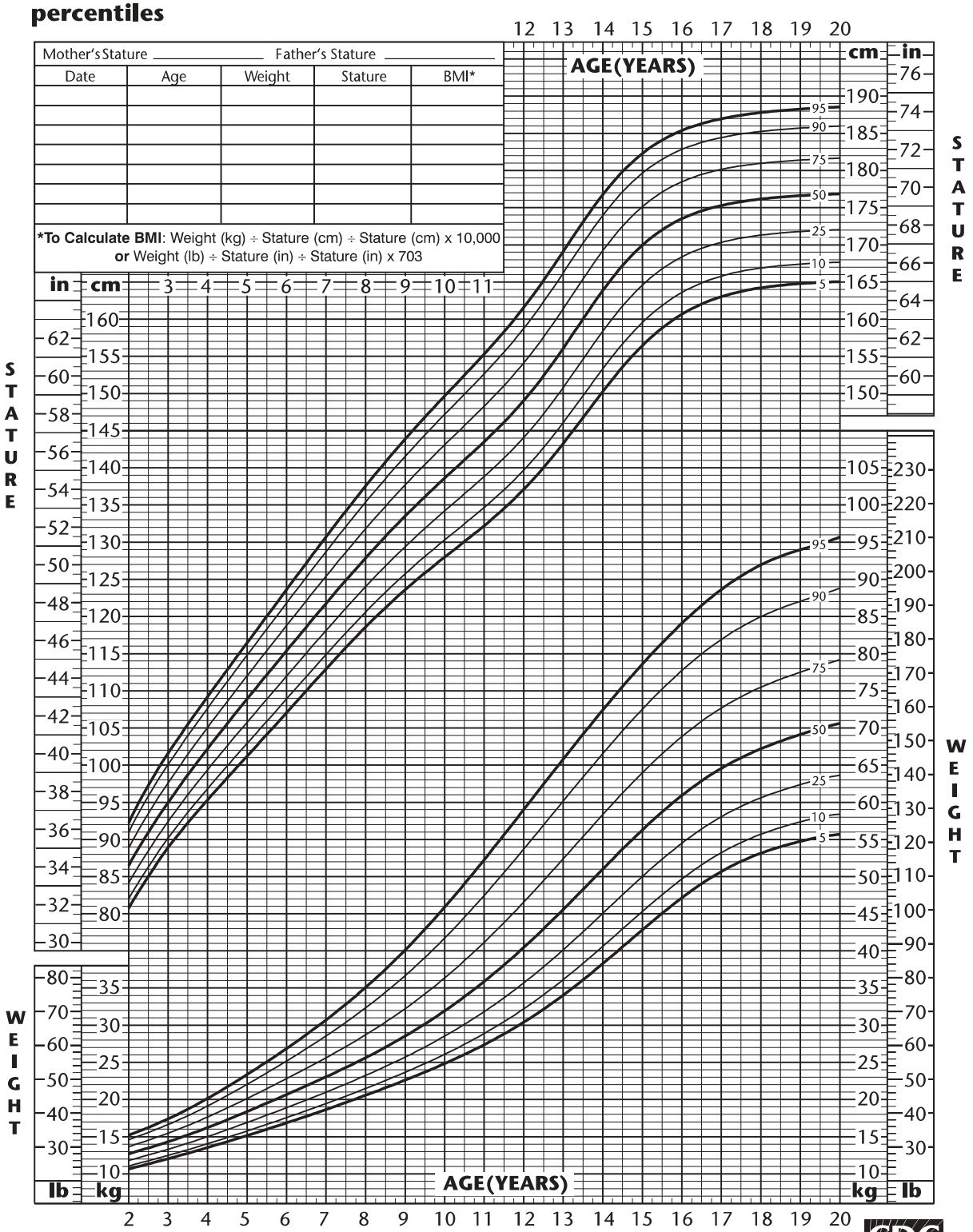
NAME _____

RECORD # _____

Mother's Stature _____ Father's Stature _____

Date	Age	Weight	Stature	BMI*

***To Calculate BMI**: Weight (kg) ÷ Stature (cm) ÷ Stature (cm) x 10,000
or Weight (lb) ÷ Stature (in) ÷ Stature (in) x 703



Revised and corrected
November 28, 2000

SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000). **http://www.cdc.gov/growthcharts**

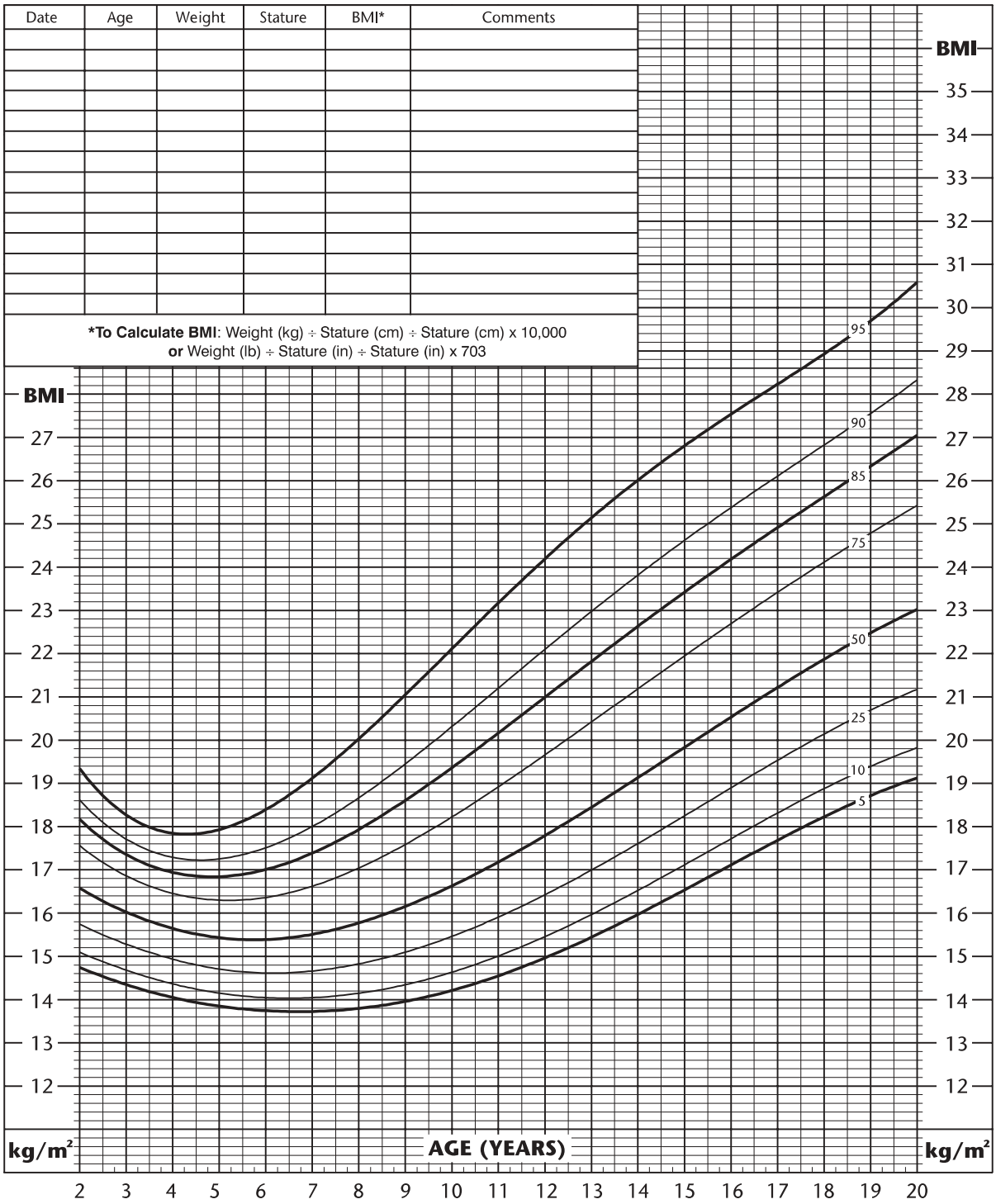
2 to 20 years: Boys
Body mass index-for-age percentiles

NAME _____

RECORD # _____

Date	Age	Weight	Stature	BMI*	Comments

***To Calculate BMI**: Weight (kg) ÷ Stature (cm) ÷ Stature (cm) x 10,000
or Weight (lb) ÷ Stature (in) ÷ Stature (in) x 703



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2 to 20 years: Girls
Stature-for-age and Weight-for-age percentiles

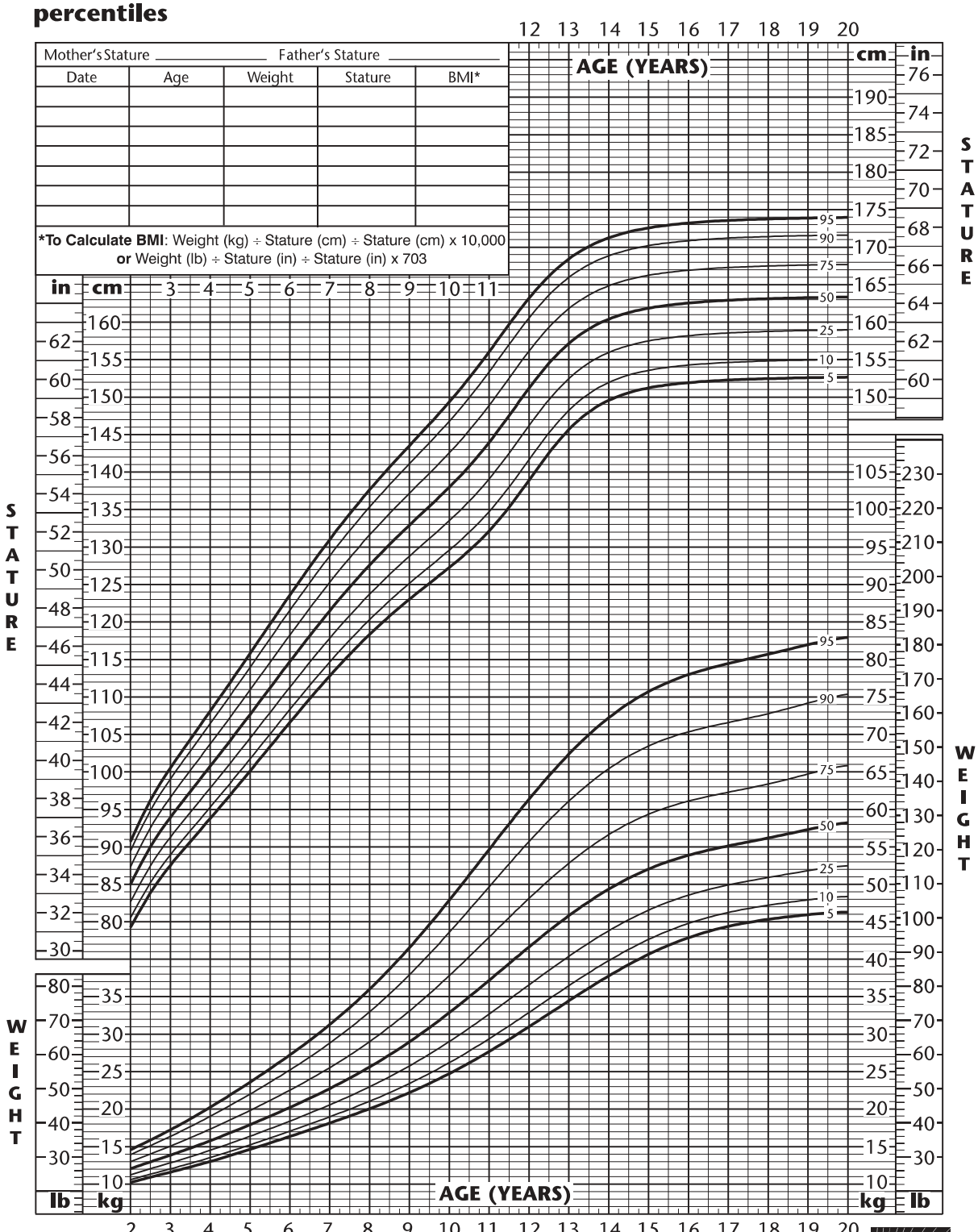
NAME _____

RECORD # _____

Mother's Stature _____ Father's Stature _____

Date	Age	Weight	Stature	BMI*

***To Calculate BMI**: Weight (kg) ÷ Stature (cm) ÷ Stature (cm) x 10,000
or Weight (lb) ÷ Stature (in) ÷ Stature (in) x 703



Revised and corrected
November 28, 2000

SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000). **http://www.cdc.gov/growthcharts**

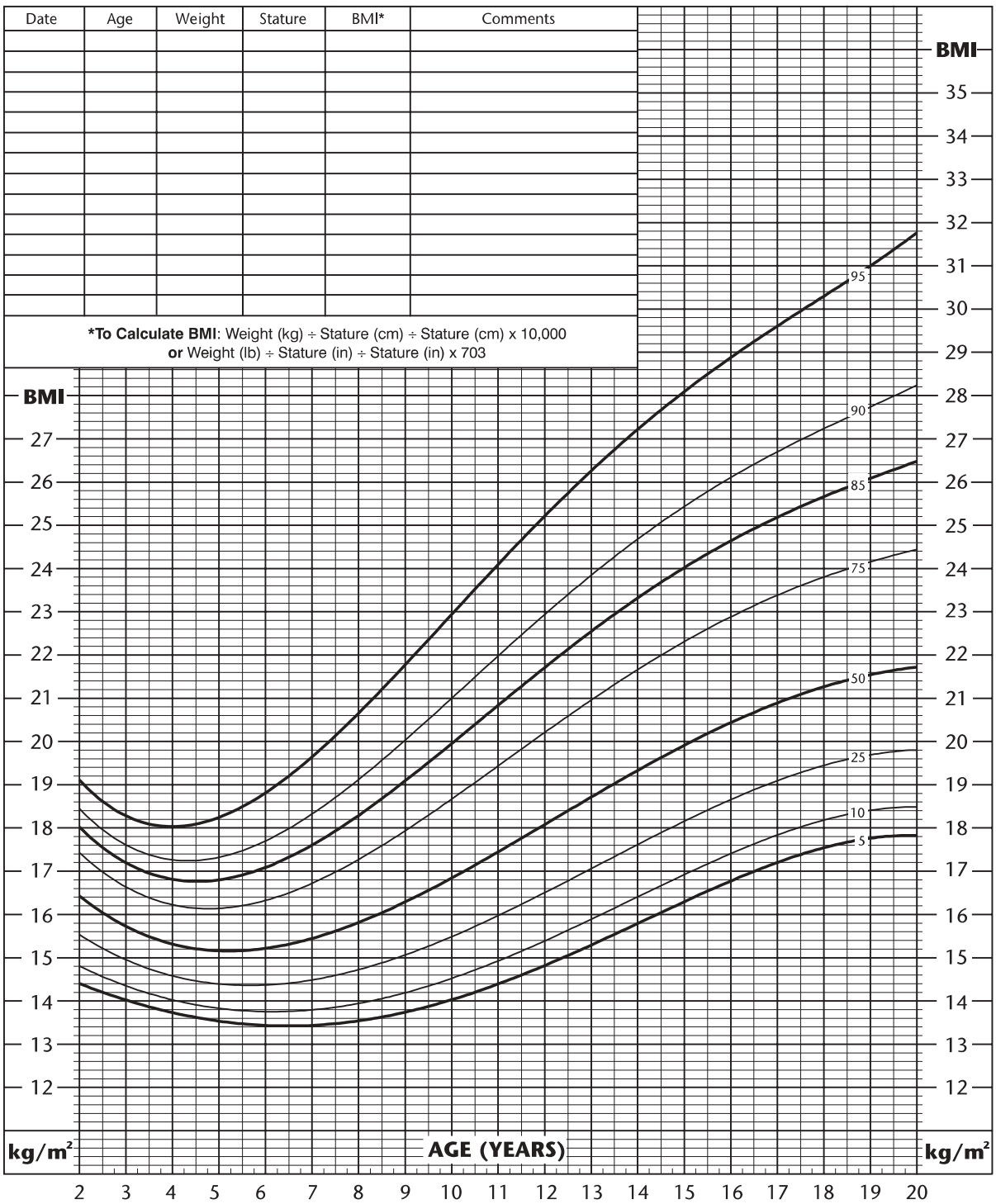
2 to 20 years: Girls
Body mass index-for-age percentiles

NAME _____

RECORD # _____

Date	Age	Weight	Stature	BMI*	Comments

***To Calculate BMI**: Weight (kg) ÷ Stature (cm) ÷ Stature (cm) x 10,000
or Weight (lb) ÷ Stature (in) ÷ Stature (in) x 703



SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000). **http://www.cdc.gov/growthcharts**

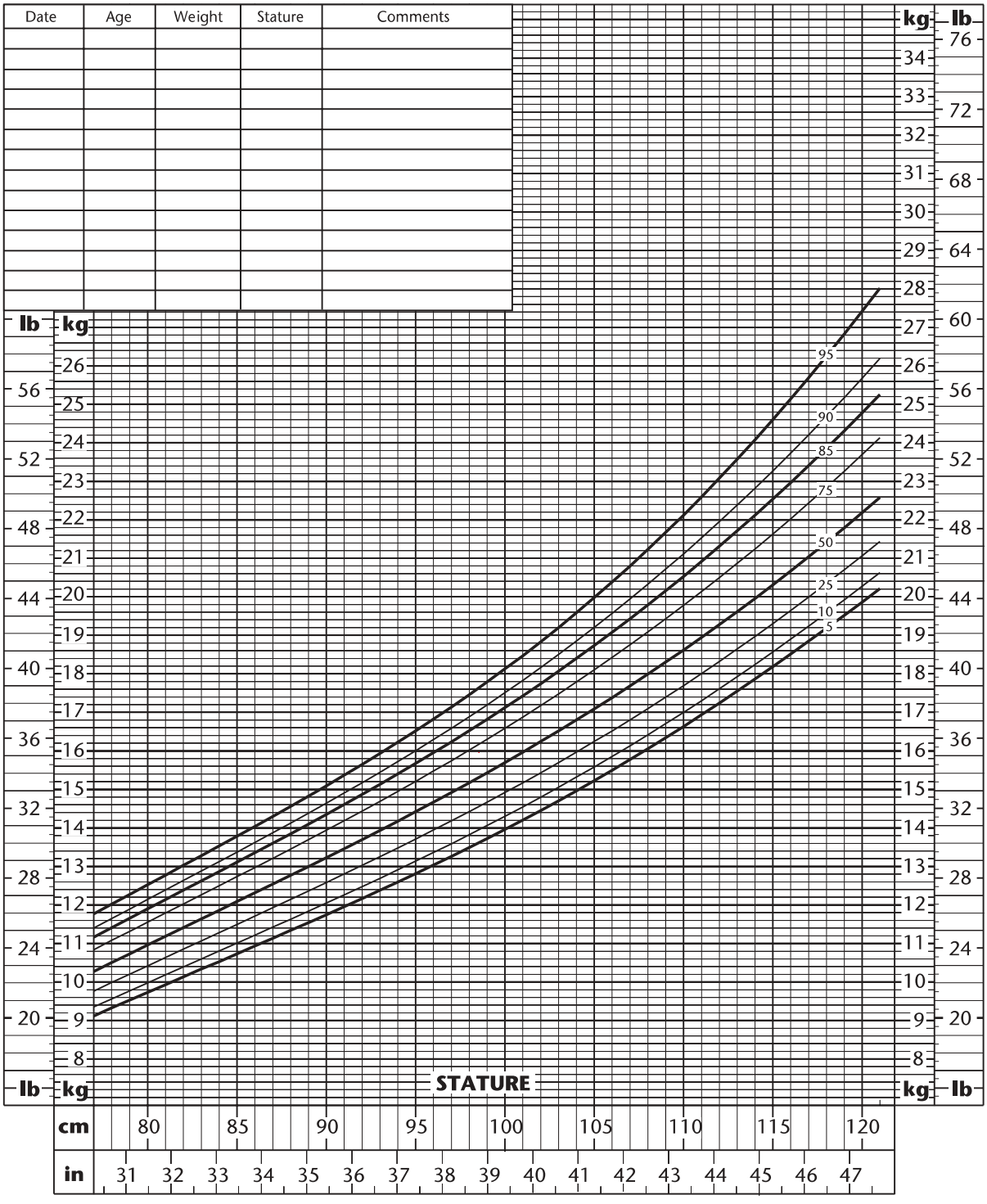
CDC

2 to 5 years: Boys
Weight-for-stature percentiles

NAME _____

RECORD # _____

Date	Age	Weight	Stature	Comments



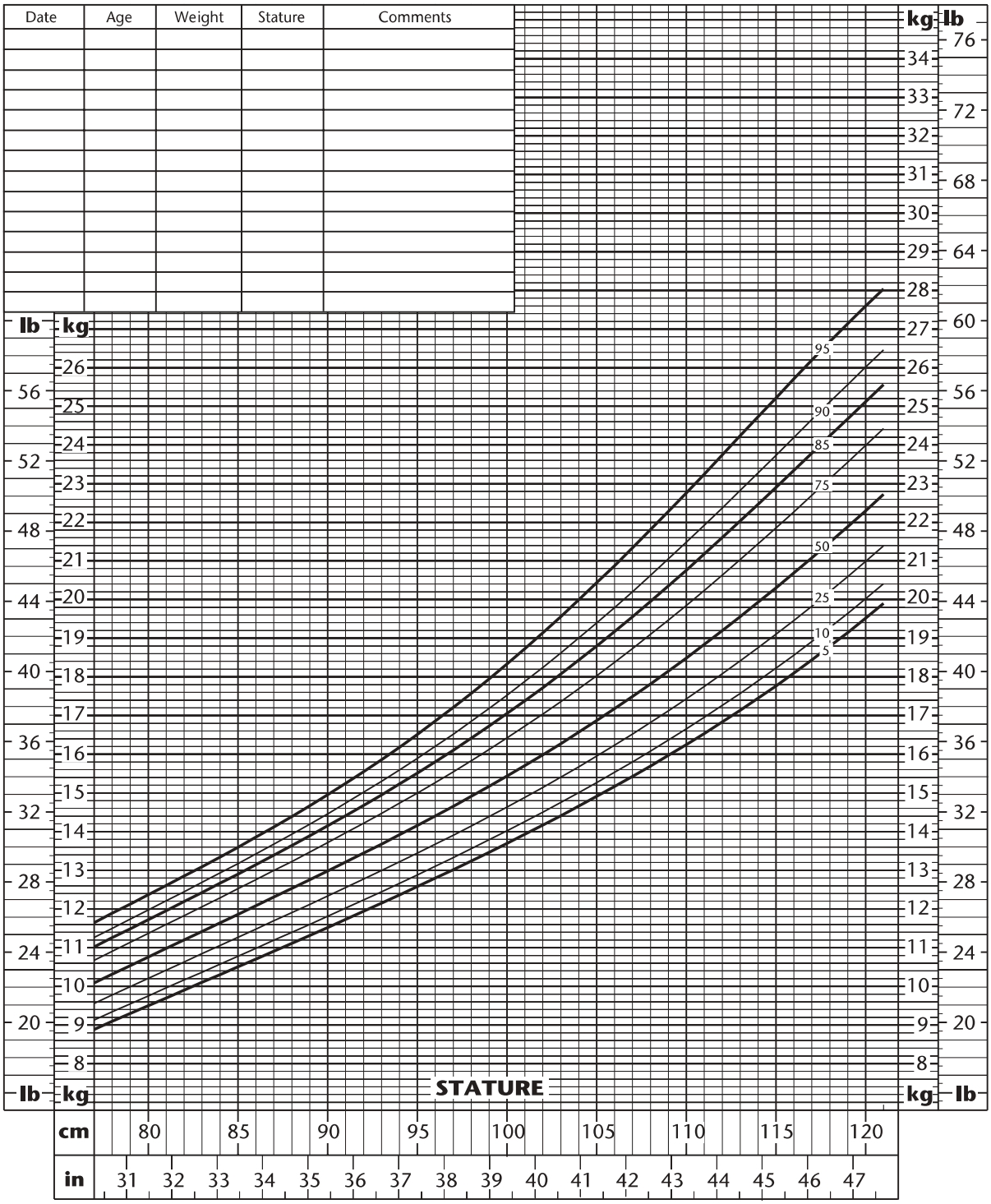
SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000). **http://www.cdc.gov/growthcharts**

2 to 5 years: Girls
Weight-for-stature percentiles

NAME _____

RECORD # _____

Date	Age	Weight	Stature	Comments



SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000). **http://www.cdc.gov/growthcharts**

The Tanner Stages

Because the onset and progression of puberty are so variable, Tanner has proposed a scale, now uniformly accepted, to describe the onset and progression of pubertal changes (Fig. 9-24). Boys and girls are rated on a 5 point scale. Boys are rated for genital development and pubic hair growth, and girls are rated for breast development and pubic hair growth.

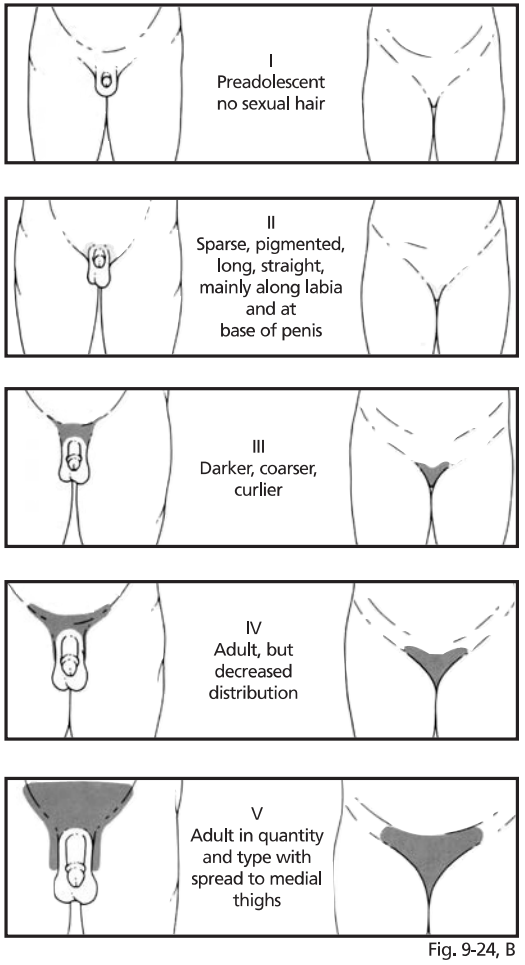


Fig. 9-24, B

Pubic hair growth in females is staged as follows (Fig 9-24, B):

- **Stage I (Preadolescent) -** Vellos hair develops over the pubes in a manner not greater than that over the anterior wall. There is no sexual hair.
- **Stage II -** Sparse, long, pigmented, downy hair, which is straight or only slightly curled, appears. These hairs are seen mainly along the labia. This stage is difficult to quantitate on black and white photographs, particularly when pictures are of fair-haired subjects.
- **Stage III -** Considerably darker, coarser, and curlier sexual hair appears. The hair has now spread sparsely over the junction of the pubes.
- **Stage IV -** The hair distribution is adult in type but decreased in total quantity. There is no spread to the medial surface of the thighs.
- **Stage V -** Hair is adult in quantity and type and appears to have an inverse triangle of the classically feminine type. There is spread to the medial surface of the thighs but not above the base of the inverse triangle.

The stages in male pubic hair development are as follows (Fig. 9-24, B):

- **Stage I (Preadolescent) -** Vellos hair appears over the pubes with a degree of development similar to that over the abdominal wall. There is no androgen-sensitive pubic hair.
- **Stage II -** There is sparse development of long pigmented downy hair, which is only slightly curled or straight. The hair is seen chiefly at the base of penis. This stage may be difficult to evaluate on a photograph, especially if the subject has fair hair.
- **Stage III -** The pubic hair is considerably darker, coarser, and curlier. The distribution is now spread over the junction of the pubes, and at this point that hair may be recognized easily on black and white photographs.
- **Stage IV -** The hair distribution is now adult in type but still is considerably less that seen in adults. There is no spread to the medial surface of the thighs.
- **Stage V -** Hair distribution is adult in quantity and type and is described in the inverse triangle. There can be spread to the medial surface of the thighs.

In young women, the Tanner stages for breast development are as follows (Fig. 9-24, C):

- **Stage I (Preadolescent) -** Only the papilla is elevated above the level of the chest wall.
- **Stage II - (Breast Budding) -** Elevation of the breasts and papillae may occur as small mounds along with some increased diameter of the areolae.
- **Stage III -** The breasts and areolae continue to enlarge, although they show no separation of contour.
- **Stage IV -** The areolae and papillae elevate above the level of the breasts and form secondary mounds with further development of the overall breast tissue.
- **Stage V -** Mature female breasts have developed. The papillae may extend slightly above the contour of the breasts as the result of the recession of the aerolae.

The stages for male genitalia development are as follows: (Fig. 9-24, A):

- **Stage I (Preadolescent)-** The testes, scrotal sac, and penis have a size and proportion similar to those seen in early childhood.
- **Stage II -** There is enlargement of the scrotum and testes and a change in the texture of the scrotal skin. The scrotal skin may also be reddened, a finding not obvious when viewed on a black and white photograph.
- **Stage III -** Further growth of the penis has occurred, initially in length, although with some increase in circumference. There also is increased growth of the testes and scrotum.
- **Stage IV -** The penis is significantly enlarged in length and circumference, with further development of the glans penis. The testes and scrotum continue to enlarge, and there is distinct darkening of the scrotal skin. This is difficult to evaluate on a black-and-white photograph.
- **Stage V -** The genitalia are adult with regard to size and shape.

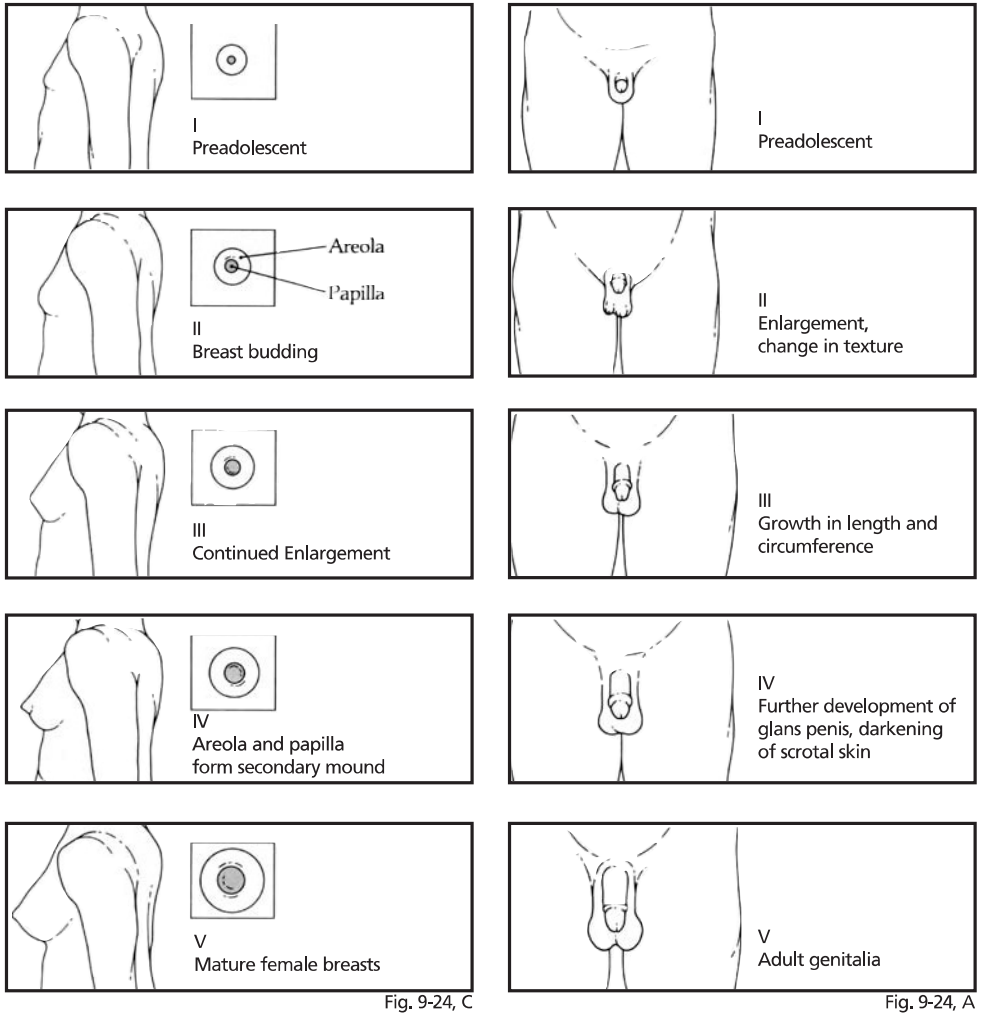


Fig. 9-24, C

Fig. 9-24, A

Source:

Reprinted with permission from Feingold, David. "Pediatric Endocrinology" In *Atlas of Pediatric Physical Diagnosis, Second Edition,* Philadelphia. W.B. Saunders, 1992, 9.16-19

Appendix (4)

Name: [redacted]	Sex: Female	Height: 151.0 cm
DOB: [redacted]	Ethnicity: Asian	Weight: 45.0 kg
		Age: 13

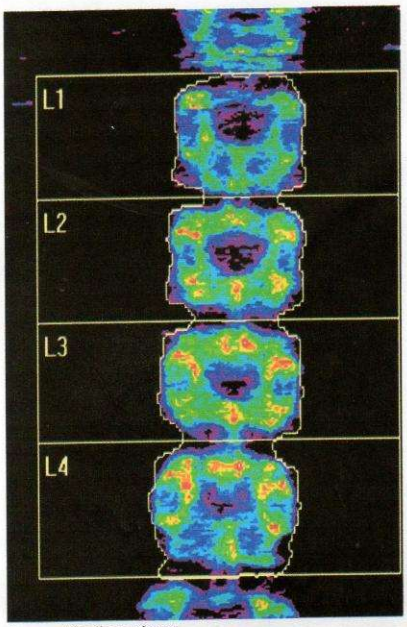


Image not for diagnostic use
k = 1.146, d0 = 46.0
116 x 127

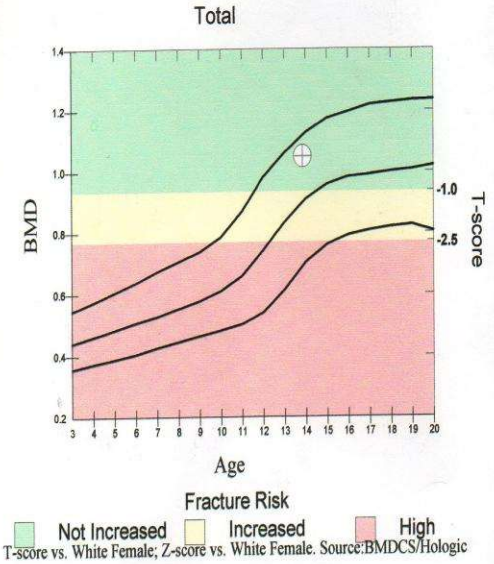
Scan Information:

Scan Date: 23 November 2010 ID: A11231004
Analysis: 23 November 2010 08:16 Version 12.7.3.1:7
Lumbar Spine
Operator: HANAA
Model: Discovery W (S/N 83903)

DXA Results Summary:

Region	Area (cm²)	BMC (g)	BMD (g/cm²)	T-score	Z-score
L1	11.49	10.98	0.956	0.3	
L2	12.09	12.53	1.037	0.1	
L3	12.36	13.74	1.112	0.3	
L4	14.50	15.69	1.082	-0.3	
Total	**50.44**	**52.94**	**1.050**	**0.0**	**1.4**

Total BMD CV 1.0%, ACF = 0.992, BCF = 0.998, TH = 6.085
WHO Classification: Normal
Fracture Risk: Not Increased



T-score vs. White Female; Z-score vs. White Female. Source:BMDCS/Hologic

الخلاصة

مرحلة البلوغ هي مرحلة مهمة جدا في عملية اكتساب الكثافة المعدنية للعظام, و من هنا تظهر أهمية تقييم الكثافة المعدنية لدى الأطفال والمراهقين. لذلك كان هدف الدراسة الحالية هو تحديد التغيرات الحاصلة في الكثافة المعدنية للعظام في مختلف مراحل البلوغ و علاقة هذه التغيرات مع العمر و مراحل البلوغ و مؤشرات النمو (الوزن و الطول و كتلة الجسم) ومع مستوى تركيز الكالسيوم والفسفور و إنزيم الفوسفاتيز القاعدي و الهرمونات الجنسية في مصل الدم, و إيجاد العامل الأساسي الذي يحدد هذه التغيرات, بالإضافة إلى ذلك تسليط الضوء على الفرق في اكتساب الكثافة المعدنية للعظام بين كلا الجنسين خلال مرحلة البلوغ.

في هذه الدراسة تم تحديد التغيرات الحاصلة في الكثافة المعدنية للعظام في منطقة الفقرات القطنية بواسطة جهاز فحص الكثافة المعدنية للعظام (مقياس امتصاص الأشعة السينية ثنائي البواعث- الدكسا) لعدد من الأطفال و المراهقين الأصحاء تم اختيارهم من مدينة الموصل في العراق, وشملت الدراسة (١٧٧) مشارك منهم (٩٦) ذكر و (٨١) أنثى تتراوح أعمارهم ما بين (١٠-20.2) سنة ,وقد أجريت الدراسة في مستشفى ابن سينا التعليمي خلال الفترة من شهر تشرين الثاني ٢٠١٠ ولغاية آذار ٢٠١١ .

تم قياس الوزن والطول وحساب كتلة الجسم، و تصنيف المشاركين حسب العمر و حسب مراحل البلوغ (و هي خمس مراحل بداية من المرحلة الأولى و هي مرحلة قبل البلوغ إلى المرحلة الخامسة و هي مرحلة البلوغ الكامل). و سحبت عينات (٥ سم^{٣}) من الدم من المشاركين و ذلك لقياس تركيز الكالسيوم الكلي والفسفور غير العضوي في مصل الدم، و قياس مستوى مؤشرات انقلاب العظام في الدم من خلال قياس مستوى إنزيم الفوسفاتيز القاعدي,

بالإضافة إلى ذلك تم قياس مستوى الهرمونات الجنسية (الهرمون الذكري– تيستوستيرون للذكور و الهرمون الأنثوي–الاسترادايول للإناث).

أوضحت الدراسة الحالية زيادة ملحوظة في الكثافة المعدنية للعظام مع تقدم العمر و تقدم مراحل البلوغ. فعند تصنيف المشاركين حسب الفئات العمرية وجد إن الإناث أكثر كثافة معدنية من الذكور في نفس الفئة العمرية بسبب دخولهن مرحلة البلوغ قبل الذكور, وان أعظم اكتساب للكثافة المعدنية في عمر ١٣ سنة بالنسبة للإناث وعمر ١٥ سنة للذكور.

وعند تصنيفهم حسب مراحل البلوغ , وجد أن الذكور أكثر كثافة من الإناث في المرحلة الثالثة فقط من مراحل البلوغ . ولوحظ في كلا الجنسين أن الزيادة الرئيسية للكثافة المعدنية للعظام بين المرحلة الرابعة و الخامسة.

و قد وجد أن مرحلة البلوغ هي العامل الأساسي الذي يحدد التغيرات في الكثافة المعدنية للعظام في كلا الجنسين بالإضافة إلى الهرمونات الجنسية والتي تتزايد بشكل ملحوظ مع تقدم مراحل البلوغ . وأن مؤشرات النمو من العوامل المهمة التي تحدد قيمة الكثافة المعدنية للعظام بالنسبة للذكور فقط و ليس الإناث.

لوحظ أن مستوى الفسفور و إنزيم الفوسفاتيز القاعدي يتغيران بصورة عكسية مع تقدم مراحل البلوغ ومع زيادة الكثافة المعدنية للعظام, أي أنهما أكثر قيمة في مراحل البلوغ المبكرة. بينما مستوى الكالسيوم لم يتغير بشكل معنوي بين جميع مراحل البلوغ. بالإضافة إلى ذلك تم استنتاج أن كل من مستوى الكالسيوم والفسفور و إنزيم الفوسفاتيز القاعدي ليست من العوامل المهمة التي تحدد قيمة الكثافة المعدنية للعظام لكلا الجنسين.

وقد تبين أن قيم الكثافة المعدنية للعظام للمشاركين هي اقل من القيم الموجودة في جهاز الدكسا بمقدار ١,٤- للذكور و ١,٢- للإناث.

إقرار المشرف

اشهد بأن إعداد هذه الرسالة جرى تحت إشرافي في فرع الفسلجة الطبية بكلية الطب في جامعة الموصل وهي جزء من متطلبات نيل شهادة الماجستير في علم الفسلجة الطبية.

التوقيع:

المشرف: أ.م.د. ساجدة سعيد الجلبي

التاريخ:

إقرار المقوم اللغوي

اشهد بأن هذه الرسالة الموسومة " تغيرات في كثافة العظم خلال مرحلة النضج" تمت مراجعتها من الناحية اللغوية وتصحيح ما ورد فيها من أخطاء لغوية وتعبيرية وبذلك أصبحت مؤهلة للمناقشة بقدر تعلق الأمر بسلامة الأسلوب وصحة التعبير.

التوقيع:

المقوم اللغوي: د. أنمار حمودي سعيد

التاريخ:

إقرار رئيس الفرع

اشهد بأن هذه الرسالة الموسومة " تغيرات في كثافة العظم خلال مرحلة النضج" قد قمت بمراجعتها و أصبحت مؤهلة للمناقشة

التوقيع:

رئيس فرع الفسلجة الطبية : أ.م.د ساجدة سعيد الجلبي

التاريخ:

إقرار رئيس لجنة الدراسات العليا

بناء على التوصيات المقدمة من قبل المشرف و المقوم اللغوي و رئيس الفرع, أرشح هذه الرسالة للمناقشة.

التوقيع:

رئيس لجنة الدراسات العليا : أ.د. عبدالجبار ياسين الحبيطي

التاريخ:

قرار لجنة المناقشة

نشهد بأننا أعضاء لجنة التقديم والمناقشة قد اطلعنا على هذه الرسالة وناقشنا الطالبة آمنة عبد الحميد عبد المجيد في محتوياتها وفيما له علاقة بها بتاريخ ٢/ ٢/ ٢٠١٢ وأنها جديرة لنيل شهادة الماجستير علوم في الفسلجة الطبية.

التوقيع
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د.فضيلة شهوان الدوسكي
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التوقيع
د. ساجدة سعيد الجلبي
عضواَ ومشرفاَ

قرار مجلس الكلية

اجتمع مجلس كلية الطب بجلسته..... والمنعقدة في / / وقرر منح الطالبة آمنة عبد الحميد عبد المجيد شهادة الماجستير علوم في الفسلجة الطبية بناءا على إكمالها متطلبات الشهادة بنجاح.

مقرر مجلس الكلية
د. احمد محمد حياوي
/ /

عميد كلية الطب
أ.د. مزاحم قاسم الخياط
/ /

((اللَّهُ الَّذِي جَعَلَ لَكُمُ الْأَرْضَ قَرَارًا وَالسَّمَاءَ بِنَاءً وَصَوَّرَكُمْ فَأَحْسَنَ صُوَرَكُمْ وَرَزَقَكُمْ مِنَ الطَّيِّبَاتِ ذَلِكُمُ اللَّهُ رَبُّكُمْ فَتَبَارَكَ اللَّهُ رَبُّ الْعَالَمِينَ))

الآية (٦٤)
سورة غافر

جامعة الموصل
كلية الطب

تغيرات في الكثافة المعدنية للعظام خلال مرحلة البلوغ

آمنة عبد الحميد عبد المجيد التحافي

رسالة ماجستير علوم
في
الفسلجة الطبية

بإشراف

د. ساجدة سعيد الجلبي

أستاذ مساعد/ فسلجة طبية

١٤٣٣ هجري

2012 ميلادي