AL-TAHADY UNIVERSITY

FACULTY OF SCIENC

DEPARTMENT OF BIOLOGY

Sirte, Libya

A STUDY ON THE EFFECT OF SEX HORMONES ON CALCIUM AND PHOSPHORUS HOMEOSTASIS

By

HODA GOMA ALSHAMY

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR MASTER DEGREE OF SCIENCE

UNDER SUPERVISION OF

DR. MAHER NAGUIB IBRAHEIM

Professor of Physiology

Faculty of Medicine

Al-Tahady University

DR. AHMED A. DABOOB

Associate Professor in Biology

Faculty of Al-Tahady University Science

(2006 – 2007)

التاريخ: 2008-5-14
الموافق: هـ
الرقم الإشاري: ت ع/ 90-113-5

الجماهيرية العربية الليبية الشعبية الاشتراكية العظمى
جامعة التحدي
سرت

Faculty of Science

Department of Biology

Title of Thesis

A Study on The Effect of Sex Hormones on Calcium and Phosphorous Homeostasis

By

Hoda Goma Alshamy

Approved by:

Dr. Maher Naghib Ibraheim
(Supervisor) .....

Dr. Ahamad Alsaghir Daboob
(Co- Supervisor) .....

Dr. Ali Mohamed Afan
(External examiner) .....

Dr. Fahima Hamed Hamam
(Internal examiner) .....

Countersigned by:

Dr. Ahmed Farag Alhgoup
(Dean of Faculty of Science)

بسم الله الرحمن الرحيم

((قل هل يستوي الذين يعلمون والذين لا يعلمون

إنما يتذكر أولوا الألباب))..

صدق الله العظيم

سورة الزمر " الآية 10 "

الإهداء

أعوام مضت وشهور من السهر والجهد المتواصل وعندما تشعر أن لحظة الصفر قد قربت وهي لحظة مناقشة الرسالة عندها يحير عقلك وفكرك لمن تهدي ثمرة هذا الإنجاز ويمر من أمامك شريط حياتك وعند أول محطة قد برزت لي عندها روح والدي العزيز رحمه الله وجعله من رفقاء الرسول في الجنة ، ومن روح أبي الطاهرة أجد نفسي قريب أحن وأمرق قلب ،وهو قلب أمي التي حتى ولو تدفق الشعر من فمي نهراً وتساقطت الكلمات من شفاي شهداً لن أستطيع أن أوفيها حقها ، وبعدها أجد نفسي وسط أخواتي (فاطمة – وأم السعد) وأخواني وأبنائهم فلا أجد ما أعبر به عنهم فلو كنت أنا الجسد فهم القلب والشرايين التي تضخ داخله وأني لأرى نفسي إلا رافعة يداي إلى الله بالدعاء لهم بطول العمر وتحقيق كل أمانيهم في الحياة من أخواني أتوجه بالقناديل والشموع التي أضاءت لي طريقي ودربي ، ولم تبخل علي بالشرح والمراجعة والنصيحة إلى أساتذتي الكرام أهدي أجمل الكلمات وأسمى آيات التقدير والاحترام ، وخلاصة هذه الرسالة ومن دون الصديق والرفيق لا نستطيع الاستمرار ،إلى صديقاتي جميعاً والآتي كن معي دائماً دافعي إلى الأمام ،وقد برهن فعلاً عن معنى الصداقة الخالصة ، وفي النهاية إليهم جميعاً أهدي ثمرة جهدي هذا مع خالص شكري وأمتناني لجهودهم معي داعية لهم بالسلامة وطول العمر في ظل أسرهم الكريمة .

اشكر الله عز وجل الذي علم الإنسان ما لم يعلم والذي أخرجنا من الظلمات إلى النور والذي هدانا إلى صراط المستقيم وما كنا نهتدي لولا أن هدانا الله .

إلى من فتح أمامي صدور المعلومات بلا حدود ولا وقت لكي يروى هذا البحث بغية أن يكون شجرة مثمرة أتقدم بأسمى معاني الشكر والتقدير والعرفان والامتنان للدكتور الفاضل ماهر نجيب إبراهيم والذي لم يبخل علي بالإرشاد والنصح المفيد ، وإلى الدكتور الفاضل أحمد السعير دبوب وذلك لما وجدته منه من رحابة وسعة صدر وحسن معاملة وتشجيع لكي يأخذ هذا البحث مساره الصحيح ولما قدمه لي من النصائح القيمة والتوصيات السديدة من اللحظة الأولى وحتى ظهور هذا البحث إلى حيز الوجود .

إلى من يعجز قلمي ويعجز لساني عن شكرهم فكانوا لي عوناً وسنداً في أحلك الأوقات والشدائد حفظهم الله ورعاهم وهم : -

الأستاذ الفاضل : - **علي إبراهيم التمامي** .

الدكتور الفاضل : - **صلاح الدين عبد الرحمن** .

كما أتقدم بجزيل الشكر والعرفان لكلاً من : -

الأخوة والأخوات موظفي كلية الطب البشري والأسنان جامعة التحدي شعبة سرت .

الدكتور : - **علي حسين بركة**

الدكتور:-**أحمد حسين سليمان**

الأخ المهندس : - **شوقي عمر الحميدي – أسامة دنده**

الأخوة والأخوات العاملين بالمختبر المركزي سبها

وإلى من لا تكفي جميع كلمات الشكر والامتنان لتوفيهم حقهم لما قدموه لي من العون والدعم : - الصديقات الوفيات

إلى كل من مد لي يد العون والمساعدة ...

No list of Abreviation	List of Abreviation	
1,25-$(OH)_2$ D_3	1,25-dihydroxycholeciferol $(OH)_2$ D	
pH	Hydrogen ion	
TNF	Tumor necrosis factor	
I (IL-I),(IL-6)	Interleukin	
La^{3+}	lanthanum	
Ca Bp	calcium-binding protein	
ATP	adenosine triphosphate	
ALP	Alkaline phosphatase	
cAMP	cyclic adenosine monophosphate	
IGF-I and IG F-II	insulin-like growth factor-I and -II	
TGF beta	transforming growth factor-beta	
DHT	dihydrotestosterone	
FGF	fibroblast growth factor	
BMD	bone metabolic density	
SHBG	sex hormone binding globulin	

No list of Abreviation	List of Abreviation	
ERT	estrogen replacement therapy	
FE	free estradiol	
TNF	tumor necrosis factor	
OVX	ovariectomized	
MPA	medroxyprogesterone	
COX-2	cyclooxygenase	
PGE2, PGF2α,	Prostaglandins	
Mg2+	Magnesium	
K+	Potassium	
TSH	Thyroid stimulating hormone	
Ca++	Calcium	
P—	Phosphorus	
T3	Tri-iodothyramine	
T4	Tetraiodothyronine	
PTH	Parathyroid hormone (parathormon)	

ABSTRACT

ABSTRACT

The present study was carried out to investigate and clarify the effect of sex hormones; testosterone in males and estrogen and progesterone in female on calcium, phosphorus and bone metabolism. It might give an idea about, the role of sex hormones in pathogenesis of osteoporosis happening in old age group of individuals of both sex.

apparently healthy(255) persons participated in this study. They were divided into 4 groups according to age. First group (= group A) included individuals under the age of 15 years old. This group represented prepubertal individuals, which have defective sex hormones. This group was subdivided into 2 subgroups; A_1 for males and A_2 for females. The second group (= group B) included individual just after pubertal age (early age groups), in which, sex hormones are highest. This group was also divided into 2 subgroups, group B_1 for males and group B_2 for females. This group included persons with age range between 20 and 35 years old. The third group (group C) included fellows of age range between 35 and 55 years old. This group represented the person in the pre and perimenstrual period, in which sex hormones start to be inadequate. This group was similarly divided into 2 subgroups, C_1 for males and C_2 for females. However, the fourth group (= group D) included individuals in the postmenopausal age (between 55 and 72 years old). In this group, sex hormones and other hormones are severely altered. Similarly, this group was subdivided into 2 subgroups, group D_1 for males and group D_2 for females.

Personal history was taken for each individual, including, his or her age, weight, smoking habit, dietary habit, muscular exercise and any past history of diseases or medication. For female individuals, menstrual history and obstetric history were taken.

Morning blood and urine samples were taken from each individual for serum analysis of calcium, phosphorus, alkaline phosphatase enzyme, testosterone hormone, estrogen and progesterone hormones, total T_3 and total T_4 and parathormone hormone. Urine sample was taken for investigating pH, urine calcium and phosphorus.

Results showed that sex hormones decrease both serum calcium and phosphorus concentration, increases serum alkaline phosphate but increase both calcium and phosphorus in urine. This was simply proven by the fact that, there was a significant elevations of in sera of both groups A and D, which include individuals of inadequate sex hormones. Situation in individuals of group D is not similar to that in individuals of group A. Several other hormones, rather than sex hormones, were altered in D group, the fact denoting that osteoporosis of old age males and females is a multifactorial process. Moreover, the parameters investigated severely change in obese non-active persons who favor fatty meals. Sex hormones appeared to have anticatabolic bony effect as shown from the activity of alkaline phosphatase enzyme.

It also shows that oestrogen and progesterone have an effect in female nearly similar to that of testosterone in male regarding calcium and phosphorus homeostasis but with a more manifested pattern in female. This explains the fact that bone loss in old women is more than bone loss in old men.

INTRODUCTION

INTRODUCTION

The capacity of specialized tissue to function in an integrated fashion in intact organism is made possible in large part by 3 systems of extracellular communication

*The nervous system which transmits electrochemical signals as two way traffic between the brain and peripheral tissue or between tissues in reflex circuits.

*The endocrine system which releases chemical mediators termed hormones into circulation for action away from their sites of origin.

*The immune system which protects the organism against external (bacteria, viruses and fungi) and internal (malignancy) threats (**Ganong, 2001**).

However together with the nervous system, the endocrinal system coordinates the function of various systems of the body as well as sharing in adaptations, according to the body needs. It is concerned with slow mechanism of regulation of metabolic processes, including all cellular reactions, and keeping internal environment and concentration of all chemicals e.g. Na^{+}, K^{+}, Cl^{-}, Ca^{++}, and P^{---} constant. The endocrinal system also controls the processes of growth and reproduction concerned with maintenance of species. The gonads are considered one of the most important endocrinal glands inside the body. They perform two physiological functions which are complementary to each other, the first is the gametogenic function which leads to the formation of sperms and ova, the second is the endocrinal function which depends on the secretion

of gonadal hormones which determine the sex and secondary sex character. Moreover, the gonadal hormones possess several effects on the general metabolism in addition to their specific effects on the target organs (**Mendel, 1999**).

Significant changes in the total body weight, water content inorganic ion concentration and metabolic activities of the target organs were noted (**Rosen, 2005**). Calcium and phosphorus are one of the very important elements in many biological processes in all mammals. Although most of calcium and phosphate are present in insoluble form in the skeleton, soluble extra skeletal calcium and phosphate are involved in several regulatory and energy related body functions. These functions include formation of bone, teething, muscle contraction, synaptic transmission, platelet aggregation, coagulation and secretion of hormones as an intracellular second messenger (**Elin *et al.*, 1994**). Less than 1% of body calcium is found in blood in extracellular fluid, and intracellularly in various soft tissues while great than 99% of the body mass calcium occurs in the skeleton. In contrast to calcium, phosphate is in non-osseous tissues in both inorganic form and as a component of various structural and functional macroglobules, including phospholipids, phosphoprotein, nucleic acids, glycogen and other intermediates of carbohydrate metabolism. These soft tissue phosphates comprise only about 15% of the total body content; the remainder is deposited as inorganic phosphate in mineral phase of bone primarily as hydroxyapatite but also as more loosely complexed amorphous forms of bone crystals (**Parfitt, 1997**). The control of extracellular calcium concentration can occur at a number of locations, the most important being bone, gut and kidney. Furthermore, the homeostasis of phosphorus is not as well controlled as that of calcium and appears to be secondary to calcium

homeostasis (**Grant *et al.*, 1990**). There are several factors involved in calcium and phosphorus homeostasis. The hormonal factor has a great role in regulation of calcium and phosphorus. Parathormone (PTH) and 1,25-dihydroxycholeciferol $(OH)_2$ D and calcitonin regulate serum of both calcium and phosphorus. The role of these hormones in regulation of calcium and phosphorus is studied by many investigators while, the role of other hormones like cortisone, thyroxine, and growth hormones are not extensively studied. On the other, hand the definite effects of gonads and male and female sex hormones need further studies to be clarified (**Guyton and Hall, 2001**). It was found that both estrogens and androgens are critical for skeletal development and maintenance. Some authors demonstrated estrogen and androgen receptor (**Vanderschueren *et al.*, 1992**). Gonadal hormones are critical for the pubertal growth spurt, and estrogen is necessary for epiphyseal closure, and deficiency of estrogen or androgen increases bone resorption in vivo, possibly by increasing local synthesis sensitivity of cytokines such as interleukin I (IL-I), Tumor necrosis factor(TNF) or Interleukin(IL-6) and to prostaglandins. Androgen can increase bone formation in vivo, the exact effect of androgens and estrogen is less clear and need to be more investigated (**Vanderschueren, 1992, Horowitz, 1997, and Ganong, 2001**). On the other hand, structure of bone is disturbed in form of osteoporosis in old ages of men and women but it appears that osteoporosis effects are more in menopausal period (**Dick, *el at* 2004**).

AIM OF THE STUDY

This study will be carried out to spotlight and clarify the effects of both androgens (male sex hormones) and estrogens and progesterone (female sex hormones) on calcium and phosphorus homeostasis in different age groups. This might help in prevention and treatment of

osteoporosis in older age especially in menopausal women and also might put an explanation for what is happening.

CHAPTER ONE

LITRATURE REVIEW

CHAPTER ONE

LITRATURE REVIEW

1.1 CALCIUM

Calcium is a very important element in many biological processes in all mammals. These processes include automaticity of nerve and muscle; contraction of cardiac, skeletal, and smooth muscle; neurotransmitter release; and various forms of endocrine and exocrine secretion (**Brook and Marshall, 2001**).

Maintenance of calcium homeostasis is important for all cells, not merely for the skeleton. During growth, calcium balance is positive. In adult life, there is an equilibrium between absorption from the gut and loss through urine (**Ganong, 2001**).

Although most of calcium and phosphate are present in insoluble from in the skeleton, soluble extraskeletal calcium and phosphate are involved in several regulatory and energy - related body functions (**Elin *et al.*, 1971; Brook and Marshall, 2001**).

1.1.1 Distribution of calcium

Less than 1 % of the body calcium is found in blood in extracellular fluid, and intracellular in various soft tissues. In addition, about 1 % of skeletal calcium is freely exchangeable with intracellular fluid. Together, these fractions are termed the miscible pool of calcium. Extracellular calcium is the principal substrate for the mineralization of cartilage and bone. But it also serves as a cofactor for many extra cellular enzymes, most notably the enzymes of the coagulation cascade, and as a source of calcium ions for a great diversity of cellular processes(**Krane, 1970 and Ganong, 2001**).

The concentration of calcium in bone is relatively constant from one species of mammal to another, although the total amount per unit of body mass varies in relation to the relative size of the skeleton (**Ganong, 2001**).

1.1.2 Calcium in the skeleton

Table (1) lists that Ninety-nine percent of total body calcium resides in bone of which 99% is located within the crystal structure of the mineral phase. Calcium in bone exists primarily in the form of small crystals, presumed to be identical to hydroxylapatites, a precise crystalline structure of calcium, phosphate and hydroxyl ions. Some calcium in bone also exists in more amorphous crystals in combination with phosphate. The normal calcium-to-phosphate ratio in bone is 1.5: 1, slightly lower than that found in pure hydroxylapatite (**Krane, 1970 and Vander *et al.*, 2001**).

A number of factors interact to regulate the balance of bone turnover. These include local factors (both chemical and mechanical) and hormonal factors (**Brook and Marshall, 2001**). In addition to parathyroid hormone and calcitonin, a variety of hormones, vitamins and other less well understood factors, influence the metabolism and turnover of bone (**Johnson ,1964 , Friedman and Gesek , 1995**).

1.1.3 Calcium in plasma

Blood taken for serum calcium determinations should be drawn in the fasting state and a tight tourniquet should not be applied for more than a few seconds, otherwise, spurious elevation of serum calcium may occur (**Grant *et al.*, 1990**). Table (2) lists the various forms in which calcium circulates in normal plasma. Slightly less than half the circulating calcium is in the form of free calcium ions. The remainder of plasma, calcium is

Table (1): Distribution of calcium and phosphorus in body tissues.

(Adapted from S.M. Krane, 1970)

	Calcium	**Phosphorus**
Total body content (g/kg fat-free tissue)	20-25	11-14
Specific tissue		
	Relative distribution (Percentage of total body content)	
Skeleton	99	85
Muscle	0.3	6
Other tissue	0.7	9

bound to serum proteins, but a small portion circulates in the form of complexes with citrate and phosphate (**Krane,1970 and Guyton and Hall, 2001**).

The normal range for total serum calcium varies slightly from laboratory to laboratory. However, it is now quite clear that the methods used to measure serum calcium must provide a normal range that does not exceed 10.5 mg/100 ml and must be reproducible with minimal deviation of 0.1-0.2 mg% from this range (**Grant *et al.*, 1990 and Conigrave *et al.*, 2000**). The upper limit of normal total serum calcium is slightly lower, by about 0.1-0.2 mg/100 in females than in males. Also normal serum calcium values in children are slightly higher than in adults (**Krane,1970 and Brook and Marshal, 2001**). In many cases of hyperparathyroidism, the serum calcium level may only exceed the upper limit of normal by as little as 0.1 mg % (**Gallapher and Wilkinson ., 1973 and Guyton and Hall, 2001**). The concentration of calcium in extracellular fluid is closely controlled. In human serum, it is normally 2.2-2.6 mmol/l. This calcium exists in three forms approximately 44 % is bound to albumin so it is not readily diffusible, about 9 % is complexed to citrate and the remainder (1.3 mmol/l). The most important fraction is uncomplexed ionized calcium (**Brook and Marshall, 2001**).

In blood, approximately 50% of total calcium is bound to proteins, mainly albumin and globulins. The ionized calcium concentration in serum is approximately 1.2 mM (5 mg/dl), and it is this fraction that is biologically active and that is tightly controlled by hormonal mechanisms because intracellular cystosolic free calcium concentrations typically are in the range of only 100 mM, a very large chemical gradient (i.e, 10.000:1), augmented by the large negative electrical potential favors calcium entry into cells through calcium channels.This gradient is maintained by the limited conductance of resting calcium channels and by

Table (2): Distribution of calcium and phosphorus in normal human. ***(Adapted from Parfitt , 1997)***

State	Concentration		Percentage of Total
	mM/l	Mg/100 ml	
Phosphorus			
Free HPO_4^-	0.50	1.55	44%
Free $H_2PO_4^-$	0.11	0.34	10
Protein bound	0.14	0.43	12
$NaHPO_4^-$	0.33	1.02	28
$CaHPO_4^-$	0.04	0.12	3
$MgHPO_4^-$	0.03	0.10	3
Total	**1.15**	**3.56**	
Calcium			
Free Ca^{++}	1.18	4.72	48%
Protein-bound	1.14	4.56	46
Complexed	0.08	0.32	3
Unidentified	0.08	0.32	3
Total	**2.48**	**9.92**	

the energy dependent extrusion of calcium into the extracellular fluid via high affinity Ca^{2+}-H^{+} ATPase and low affinity sodium calcium (Na^{+}-Ca^{2+}) exchangers **(Elin *et al.*, 1971 and Conigrave *et al.*, 2000)**.

As almost one half the calcium in serum is bound to protein-chiefly to albumin, altered albumin concentrations correspondingly alters the protein bound serum calcium, a change that is reflected on the total serum-calcium levels. Alternations in serum globulin do not change total serum calcium concentrations significantly. For example, total serum calcium is frequently low in conditions usually associated with hypoalbuminaemia such as nephritic syndrome and hepatic cirrhosis **(Brown *et al.*, 1998)**. Although albumin is the principal calcium-binding factor in plasma, but other proteins, including globulins also bind significant quantities of the ions **(Krane,1970 and Ganong, 2001)**. Calcium binding by proteins is pH-dependent. Decreased binding occurs at acid pH **(Krane,1970., Oster *et al.*, 1978 and Guyton and Hall, 2001)**.

Since the extent of Ca^{2+} binding by plasma proteins is proportionate to the plasma protein level, it is important to know the plasma protein level when evaluating the total plasma. Plasma ionized calcium can be measured by use of a calcium-sensitive electrode. Other electrolytes and pH affect the Ca^{2+} level **(Guyton and Hall, 2001)**.

Thus, symptoms of tetany appear at much higher total calcium level if the patient hyperventilates, increasing plasma pH . Plasma proteins are more ionized when the pH is high, providing more protein anion to bind with Ca^{2+} **(Kenneth, 1998 and Ganong, 2001)**. Changes in plasma hydrogen ions concentration can influence the degree of calcium binding to plasma proteins with acidosis; less calcium is bound to plasma

proteins. Conversely, in alkalosis, a greater amount of calcium is bound to the plasma proteins. Therefore, Figure (1) shows patients with alkalosis are more susceptible to hypocalcemic tetany **(Guyton and Hall, 2001).**

As non-proteins bound serum calcium can pass through the capillary wall into interstitial fluid or through a membrane with ultrafine pores such as cellophane, this fraction is frequently referred to as the "ultrafiltrable" calcium **(Conigrave *et al.*, 2000 and Guyton and Hall, 2001).** It is to be noted that the ultrafiltrable calcium is the fraction which reflects the level of ionized calcium in the blood more closely than the total calcium level. The major portion of the ultrafiltrable serum calcium is ionized, but a small fraction presists as soluble and diffusible. But un-ionized complexes with phosphate, citrate, and bicarbonate **(Krane,1970 and Kenneth, 1998).**

The free ionized calcium in the body fluid that is a vital second messenger and is necessary for blood coagulation, muscle contraction, and nerve function. A decrease in extracellular Ca^{2+} at the myoneural junction inhibits transmission, but this effect is overbalanced by the excitatory effect of a low Ca^{2+} level on the nerve and muscle cells. The result is hypocalcemic tetany **(Ganong, 2001).**

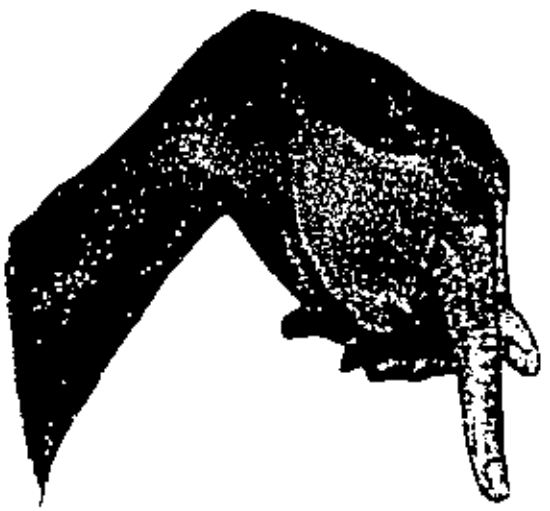


Fig. 1 Characteristic tetany of the hand.

1.1.4 Calcium in extraskeletal cells

The concentration of extracellular fluid calcium is $2.5x10^{-3}$ M, the concentration of intracellular calcium, specially in the cytosol, although difficult to measure, has been estimated to be much lower than that present in extracellular fluid, less than 10^{-6} M (**Borle,1967, Rasmussen *et al.*,1976 and Conigrave *et al.*, 2000**).

However, that total cellular content of calcium is sufficient, if uniformly distributed, to raise the cellular calcium concentration considerably, to $20x10^{-3}$ M, close to that of extracellular fluid, presumed that, cells contain an active calcium pump analogous to other cellular ion transport pumps. Alternatively, part of the regulation of cellular calcium content may involve membrane permeability. It is known that the permeability of the plasma membrane to calcium does not change dramatically with various tropic influences, such as the action of hormones on target cells (**Rasmussen *et al.*, 1976 and Guyton and Hall, 2001**). The cells must pump out Ca^{2+} and keep it at a low intracellular concentration to maintain a high phosphate concentration but avoid crystallization of calcium phosphate, (**Kenneth, 1998**).

1.1.5 Absorption of calcium

In adult, an equilibrium is reached between absorption and excretion. Dietary intake provides about 1 g of calcium. Intestinal secretion adds another 7 mmol of calcium to the contents of the intestinal lumen, and only part of the calcium in the lumen is absorbed into the bloodstream. Absorption is balanced by the renal loss of calcium, which ranges between 3 and 7 mmol in 24h (**Brook and Marshall, 2001**) . A variety of factors, including the actual dietary intake, previous dietary history, age, general state of overall calcium balance, and intake of

vitamin D, influence the efficiency of absorption (**Krane,1970; Wasserman and Taylor , 1976 and Goussous *et al.*, 2005**).

Calcium homeostasis depends on a balance between dietary intake, urinary and fecal losses, and exchanges with the osseous tissue. The balance between osseous deposition and resorption is regulated by two hormones calcitonin and parathyroid hormone (**Kenneth, 1998**).

Although a large share of calcium excretion occurs in the feces only about 10% of ingested calcium normally is absorbed from the intestinal tract, and remainder is excreted in feces. Under certain conditions, fecal calcium excretion can exceed calcium ingestion because calcium can also be secreted into the intestinal lumen. Therefore, the gastrointestinal tract and the regulatory mechanisms that influence intestinal calcium absorption and secretion play a major role in calcium homeostasis (**Guyton and Hall, 2001**).

The net intestinal absorption of calcium equals the dietary calcium minus the quantity lost in feces. Each day this amounts reach to 200 mg, which is roughly equivalent to the daily loss in the urine, so, the net intestinal calcium absorption = dietary Ca (800 mg) - fecal Ca (600 mg) = 200 mg. However, it is obvious that the amount of calcium actually absorbed by the gut each day exceeds this figure by an amount equal to the quantity of calcium in intestinal juices. Thus, the total intestinal absorption of calcium dietary Ca (800 mg) + Ca in intestinal juices (600 mg) - fecal Ca (600) = 800 mg (**Krane, 1970 and Ganong, 2001**).

1.1.6 Mechanisms of intestinal calcium absorption

There is abundant evidence indicating that passive permeability or simple diffusion also has a quantitatively important role in the absorption

of calcium and this process is also influenced or regulated by vitamin D, as in active transport **(Papworth and Patrick ,1970)**.

Extensive study by **Wasserman and Taylor** in 1976, has established two general mechanisms in intestinal calcium absorption, both active transport and diffusion related process are involved. In the presence of adequate concentrations of vitamin D, calcium is transported from mucosa to serosa against a concentration gradient **(Potts *et al.*, 1974)**. However, in situ perfusion studies have also shown concentration gradients indicating active calcium transport. A requirement for metabolic energy in these mechanisms of calcium transport has been shown as expected for an active transport process **(Potts *et al.*, 1974; Wasserman and Taylor, 1976 and Ganong, 2001)**.

Under normal dietary conditions, calcium intake is in the range of 700 to 900 mg daily. Approximately 30-35% of this calcium is absorbed; however, losses from intestinal secretion of calcium lead to a net daily uptake of only approximately 200 mg **(Van, 1987 and Brown *et al.*, 1998)**.

Although vitamin D is the major hormonal determinant of intestinal calcium absorption, the bioavailability of mineral ions in the intestinal lumen may be affected by a number of local factors and dietary constituents **(Conigrave *et al.*, 2000)**.

Absorption of calcium and magnesium is impaired by bile salt deficiency, unabsorbed free fatty acids in steatorrheic states, and high dietary content of fiber or phytate. Gastric acid is needed to promote dissociation of calcium from anionic components of food or therapeutic preparations of calcium salts **(Elin *et al.*, 1971 and Karbach, 1992)**.

Calcium is thought to be absorbed by three pathways (1) the transcellular route, (2) vesicular calcium transport, and (3) para cellular transport. The first two pathways are dependent on 1,25-dihydroxycholeciferol $(OH)_2 D_3$. Although the necessity of vitamin D for para cellular calcium absorption remains controversial, substantial evidence exists that the hormone enhances this pathway as well **(Wasserman and Fullmer, 1983 and Karbach, 1992)**.

The existence of passive absorption process is deduced from several lines of evidence. Study of calcium absorption as a function of calcium concentration in the intestinal fluid indicates that the rate of mucosal to serosal flux of calcium varies as the calcium concentration increases, even to very high levels, without evidence of saturation in intestinal lumen. An active transport process would, of course, be saturable **(Papworth and Patrick, 1970 and Ganong, 2001)**.

Wasserman and Taylor (1976) studied the concentration of calcium in intestinal lumen required to equal the electrochemical potential difference between the concentration of calcium in the lumen and in blood. They estimated that above a luminal calcium concentration primarily by the diffusional process stated in other terms. This analysis leads to the conclusion that under conditions of calcium intake or in regions of the intestine where calcium concentration is high. Absorption is diffusion-limited, and the passive permeability process predominates. In other regions of the intestine or at times when luminal calcium concentration is lower than approximately 6 millimol, the active transport process assumes a preponderant role **(Wasserman and Fullmer ,1983 and Karbach, 1992)**.

1.1.7 Intestinal sites involved in calcium absorption

Studies by **Wassermann and Taylor , (1976)** clarified that the epithelial cells of the intestine contain multiple specialized structures important in the special function of controlled absorption of calcium between cells and restrict absorption to direct transcellular transport **(Wasserman and Taylor, 1976 and Guyton and Hall, 2001).**

Other previous studies by **Wasserman et al., (1973 and 1976)** however, employing lanthanum (La^{3+}) have suggested that there may be a paracellular path for calcium absorption. In some sites or under some conditions the tight junctions may be leaky. On the surface of the microvilli is a mucopolysaccharide (glycocolyx) coat. The transcellular transport of calcium involves movement through this mucoplysaccharide coat then through the microvilli on the luminal membrane surface. Multiple subcellular organelles and finally a pump mechanism operating at the lateral or basal membranes of the cells adjacent to the capillary bed represent additional components in the pathway of overall transepithelial transport of calcium from lumen to blood. With regards to specific regions of the small intestine. duodenum. jejunum. or ileum. estimates of the intraluminal calcium concentration in animal species have indicated a much higher concentration of calcium in the ileum than in the duodenum and jejunum **(Cramer, 1965 and Fullmer. 1992).**

Although it has been known that the duodenum is the region of most efficient calcium absorption a variety of lines of evidence by **(Cramer and Copp., 1959 and Cramer, 1965)** . They suggest that the greatest proportion of intestinal calcium absorption is accomplished in the ileum - especially under natural conditions when calcium is ingested along with solid food. The active transport process seems to predominate in duodenum and jejunum. The importance of duodenum and upper

regions of the jejunum becomes greatest when the calcium content of the diet is low (**Kimberg *et al.*, 1961**).

1.1.8 Controlling influences in calcium absorption

1.1.8.1 Vitamin D, calcium transport proteins, and adaptation of absorption.

The dependency of the active transport mechanism on presence of adequate vitamin D metabolites has been abundantly demonstrated (**Schacter, 1963; Potts *et al.* ,1974, Wasserman and Taylor, 1976**). The major controlling influence on the efficiency of intestinal calcium absorption is 1,25- dihydroxy vitamin D3 (1.25-$(OH)_2$-D_3), the presumed sole active metabolite of vitamin D. The critical role vitamin D in the enhancement of intestinal absorption of calcium has been extensively studied by many workers for many years (**Schacter, 1963 and Guyton and Hall, 2001**).

It is not yet clear whether vitamin D independently affects both the calcium pump mechanism involved in active transport and some critical component(s) involved in passive diffusional absorption of calcium (**Harrison and Harrison ., 1961**). **Wasserman and Taylor (1976)** reported that vitamin D can direct synthesis of a calcium binding protein in intestinal mucosa. This protein may play an important role in the active transport mechanism. Other studies have indicated that vitamin D plays an important role in the second process, or diffusional-permeability absorptive pathway for calcium (**Potts *et al.*, 1974 and Wasserman and Fullmer, 1983**)

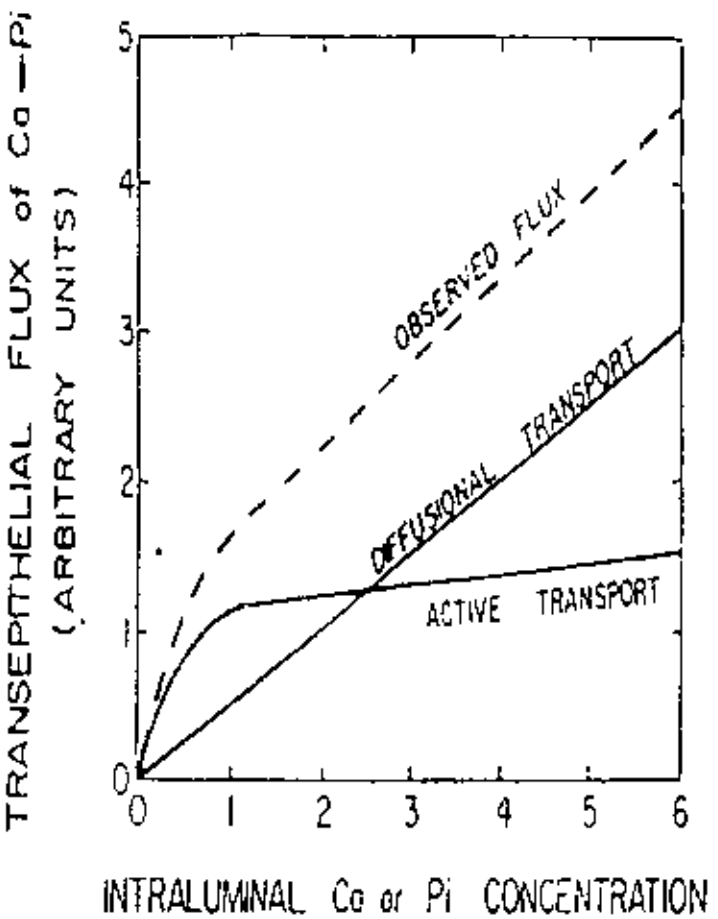


Fig. 2 The active and passive components of intestinal mineral ion.

The protein has been isolated from several animal species including humans **(Guyton and Hall, 2001)**, the concentration of the binding protein changes in response to physiological and nutritional variables **(Wasserman and Taylor, 1976)**. second protein, a calcium dependant ATP are present in the intestinal brush borders has been described and linked in function to the calcium binding protein. The synthesis of the ATPase is also under control of vitamin D **(Kowarski and Schacter, 1973; Ganong, 2001 and Guyton and Hall, 2001)**. Other studies have pointed to a third protein involved in calcium transport, namely brush border alkaline phosphates **(Ganong, 2001)**. Certain studies indicated that the ATPase and alkaline phosphatase may be the same protein **(Wasserman and Taylor, 1976 and Somjen et al., 1989)**.

Recent studies clarified that other effects of 1,25-dihydroxy-choleciferol that might play a role in promoting calcium absorption are the formation of (1) a calcium stimulated ATPase in the brush border of epithelial cells and (2) an alkaline phosphatase in the epithelial cells. The precise details of all these effects are nuclear(**Guyton and Hall., 2001**).

Good correlations have been established between the calcium-binding protein (Ca Bp) and intestinal calcium transport. Synthesis of protein appears to be dependant on vitamin D. A correlation exists between Ca2+ absorption rates and the intestinal concentration of calcium binding protein (Ca Bp). When calcium absorption rates are high, as in vitamin D treated animals, the content of calcium-binding protein is high **(Wasserman et al., 1973; Wasserman and Taylor, 1976 and Ganong, 2001)**.

When calcium absorption is depressed, as in rickets, anticonvulsant therapy or diabetes, the content of binding protein in intestine falls.

Although vitamin D is an absolute requirement for the adaptive mechanisms a potential role for other factors has been investigated **(Kimberg et al., 1961 and Goussous et al ., 2005).**

The previous studies by **Armbrecht (1986)** to determine whether there are changes in intestinal Ca++ and P-3 uptake with age and whether the regulation of Ca++ and P-3 uptake change with age experiments were performed in male fischer 344 rats aged 2–3 months (young), 12-14 months (adult) and 22-24 months (old) Ca++ and P-3 uptake were measured simultaneously by incubating everted intestinal sacs in a buffered salt solution containing radiolabeled 0.25 mM Ca+2 and 1.0 mM P for 15 min, Ca+2 uptake declined by over 50% with age in the duodenum and P-2 uptake showed a similar decline in both duodenum and jejunum. The biggest decrease was seen between the young and adult age groups. These decreases in uptake were paralleled by decreases in serum 1,25- dihydroxyvitamin D. With age, administration of 1, 25-dihydroxyvitamin D-3 increased Ca+2 uptake by 50-65% in the duodenum and increased P-3 uptake by 85-120% in the duodenum and jejunum of both young and adult rats. Although 1,25- dihydroxyvitamin D-3 increased uptake by about the same percentage in each age group. the maximal uptake was much greater in young than in adult. Feeding a low Ca+2 diet increased duodenal Ca+2 uptake by 68% and increased serum 1,25-dihydroxyvitamin D over 2-fold in young rats. There was no significant increase in either parameter in adult rats fed a low-ca diet. However, duodenal P uptake was stimulated by a low Ca+2 diet by 87 % in young rats and 51% in adult rats. These results demonstrate that there is an age-related decline in Ca+2 and P-3 uptake by intestinal mucosa in addition, there is decreased capacity of 1,25- dihydroxyvitamin D-3 and a low Ca-2 diet to stimulate intestinal uptake in the adult.

1.1.8.2 Other factors influencing intestinal calcium absorption

Parathyroid hormone, calcitonin, phosphate, certain cations, cortisol. Several drugs and certain sugars and amino acids have all been shown to influence intestinal calcium absorption with the probable exception of parathyroid hormone (**Potts *et al.*, 1974; Wasserman and Fullmer, 1983 ., Boland *et al.*, 1990 and Massey and Whiting ., 1993**).

The parathyroid hormone markedly increases the efficiency of intestinal calcium absorption. The bulk of evidence indicates that the effect of parathyroid hormone is indirect through the stimulation of increased 1,25- dihydroxy vitamin D (**Wasserman and Taylor, 1976 and Guyton and Hall, 2001**). PTH also increases the formation of 1,25-dihydroxycholeciferol, the physiologically active metabolite of vitamin D. It increases Ca^{2+} absorption from the intestine (**Ganong, 2001 and Vander *et al.*, 2001**).

High intakes of phosphate depress intestinal calcium absorption. Since there is abundant evidence that the calcium absorptive and phosphate pathways or transport systems are separate, the most likely explanation of the effect of high-phosphate diet on intestinal calcium absorption is that relatively insoluble calcium-phosphate complexes are formed, thereby rendering the calcium less available for transepithelial uptake (**Wasserman and Taylor,1976 and Brook and Marshall, 2001**).

Sodium and potassium can all be shown to inhibit calcium absorption when present in high concentrations. Alternatively, low concentration of sodium increases intestinal calcium transepithelial uptake in vitro. It is not clear that the effects attributable to sodium and potassium are normally operative at physiological concentrations of these two ions (**Martin and Deluca ,1969 and Nordin *et al.*, 1993**).

PTH also increases renal tubular reabsorption of calcium. At the same time it diminishes phosphate reabsorption. Moreover it increases the rate of reabsorption of magnesium ions and hydrogen ions while it decreases the reabsorption of sodium **(Guyton and Hall, 2001)**.

Cortison or other glucocorticoids in high doses has been known for many years to inhibit the sufficiency of calcium absorption **(Potts *et al.*, 1974 and Wasserman and Taylor,1976)**.

Lactose and other sugars such as mannose, xylose and related compounds (but not glucose) exert an inhibitory effect on intestinal calcium absorption **(Wasserman and Taylor,1976)**.

It is not clear that under normal conditions these effects are important in limiting intestinal calcium absorption. Certain amino acids such as lysine and arginine can inhibit intestinal calcium absorption **(Penzes *et al.*, 1973 and Karbach, 1992)**. The mechanism of the amino acid effect, like that of lactose and sugars, is not settled, but certain evidence suggests that both sugars and amino acids may act to chelate calcium **(Wasserman and Taylor,1976 and Kumar, 1994)**.

Ethanol in high doses both in animals and in humans can be shown to inhibit intestinal calcium absorption **(Krawitt, 1973 and Pointon *et al.*, 1979)**.

In diabetic animals, the calcium binding protein is depressed in intestinal tissue. This correlates with earlier observations that calcium absorption is reduced in diabetic animals **(Barneet and Wasserman., 1973)**. In addition, analogues of vitamin D have been shown to synergize with cyclosporine in preventing rejection of transplanted pancreatic islet cells in murine module **(Krawitt, 1973 and Kumar, 1994)**.

Intestinal absorption of calcium is diminished by alkalinization of gut contents and by dietary factor such as oxalate and phytate (hexaphosphoinositol, a constituent of the bran), which form insoluble salts or complexes with calcium **(Barneet and Wasserman., 1973).**

One analogue, 22-oxacalcitriol, suppresses PTH synthesis and secretion in rats at doses that stimulate intestinal calcium absorption less than that caused by 1.25 $(OH)_2$ D_3 **(Brown *et al.*, 1998).**

1.1.9 Excretion of Calcium

The intestinal secretion of calcium or the endogenous fecal calcium excretion does not seem to be an important route of calcium excretion. Losses of calcium in sweat are usually minor. Only when there is sustained loss of sweat excretion occurs via the skin which can be considered significant, reaching values of 100-200 mg/day **(Bijvoet *et al.*, 1977 and Guyton and Hall, 2001).**

By far the principal route of excretion is therefore renal. Excretion via the kidneys in a normal subject may be as great as 400 mg/24 hours, although it is usually lower **(Bijvoet *et al.*, 1977 and Friedman and Gesek., 1995).**

As with other substances in the body, the intake of calcium must be balanced with the net loss of calcium over the long term. Unlike ions such as sodium and chloride, however, a large share of calcium excretion occurs in feces, only about 10 percent of ingested calcium normally is absorbed from the intestinal tract, and the remainder is excreted in feces. Under certain conditions, fecal calcium excretion can exceed calcium ingestion because calcium can also be secreted into the intestinal lumen.

Therefore, the gastrointestinal tract and the regulatory mechanisms that influence intestinal calcium absorption and secretion play a major role in calcium homeostasis **(Guyton and Hall, 2001)**.

Clearly one of the important factors controlling renal excretion of calcium is simply the total amount of filtered calcium **(Bijvoet *et al*, 1977 and Guyton and Hall, 2001)**. When calculations are made of the amount of non-protein bound calcium filtered daily by the glomerulus, it is seen that 7-10 gm/24 hours may be filtered. Since urinary calcium excretion normally ranges only between 135 and 400 mg/24 hours, it is clear that about 96 to 99% of filtered calcium is normally reabsorbed. Theoretical treatments of calcium excretion lead to the conclusion that below 9.5 mg/100 ml of total serum calcium or 5.8 mg/100 ml of ultra filterable calcium in serum, reabsorption exceeds 99% **(Bijvoet *et al.*, 1977 and Fullmer, 1992)**. Because calcium is both filtered and reabsorbed in the kidneys but not secreted, the rate of renal calcium excretion is calculated as

Renal calcium excretion = calcium filtered – calcium reabsorbed.

Only about 50% of the plasma calcium is ionized, with the remainder being bound to the plasma proteins. Therefore, only about 50% of the plasma calcium can be filtered at the glomerulus. Normally, about 99 per cent of the filtered calcium is reabsorbed by the tubules, with only about 1% of the filtered calcium being excreted. About 65% of the filtered calcium is reabsorbed in the proximal tubule, 25 to 30 per cent is reabsorbed in the loop of Henle, and 4 to 9 percent is reabsorbed in the distal and collecting tubules. This pattern of reabsorption is similar to that for sodium **(Bijvoet *et al.*, 1977 and Guyton and Hall, 2001)**.

As is true with the other ions, calcium excretion is adjusted to meet the body's needs. With an increase in intake, there is also increased renal calcium excretion, although much of the increase of calcium intake is eliminated in the feces with calcium depletion, calcium excretion by the kidneys decreases as a result of enhanced tubular reabsorption **(Guyton and Hall, 2001)**.

If blood calcium concentration falls to hypocalcemic level, this efficiency of reabsorption can rise to essentially 100% when serum protein concentration is normal, this disappearance of calcium from urine occurs when blood calcium concentration fall below 7.5 mg/100 ml **(Bijvoet *et al.*, 1977; Kenneth, 1998)**.

In the proximal tubule, calcium reabsorption usually parallels sodium and water reabsorption. Therefore, in instances of extracellular volume expansion or increased arterial pressure. Both of which decrease proximal sodium and water reabsorption **(Kenneth, 1998)**.There is also reduction in calcium reabsorption and, consequently, increased urinary excretion of calcium. Conversely, with extracellular volume contraction or decreased blood pressure, calcium excretion decreases primarily because of increased proximal tubular reabsorption **(Guyton and Hall, 2001)**. It appears that calcium and sodium ions share a common pathway of active transport in the proximal tubule **(Bijvoet *et al*, 1977 and Massey and Whiting, 1993)**.

Although there is previous evidence which suggests that parathyroid hormone may inhibit proximal tubular calcium transport, other evidences suggest that parathyroid hormone does not affect proximal calcium absorption **(Clark and Dantzler ., 1972; Bijvoet *et al*, 1977 and Ganong, 2001)**.

One of the primary controllers of renal tubular calcium reabsorption is PTH. With increased levels of PTH, there is increased calcium reabsorption in the thick ascending loops of Henle and distal tubules, which reduces urinary excretion of calcium. Conversely, reduction of PTH promotes calcium excretion by decreasing reabsoprtion in the loops of Henle and distal tubules **(Bijvoet *et al* .,1977 and Guyton and Hall, 2001)**.

PTH augments synthesis of 1,25- $(OH)_2$ D_3 by the proximal tubules of the kidney, and 1,25- (OH) D_3 then acts direct upon enterocytes to increases active transcellular transport of calcium. Enhanced intestinal calcium absorption is quantitatively the most important response to calcium deprivation. Renal tubular calcium reabsorption is increased by PTH, an effect that is enhanced by increased 1,25- $(OH)_2$ D_3 – stimulated expression of calbindin – D25 in the distal tubules. Calcium reabsorption is also enhanced directly by any tendency to hypocalcemia, which is detected by calcium-sensing receptors in Henle's loop (and possibly also in the distal nephron) that control transepithelial calcium movements independent of PTH or 1,25- $(OH)_2$ D_3 **(Bijvoet *et al.*, 1977).**

It is to be noted that, the implications concerning calcium excretion are several. First, as serum calcium falls below the threshold value (9.5 mg/100 ml) urinary calcium excretion falls rapidly, which is homeostatic. Conversely, when serum calcium rises, calcium is rapidly excreted. Furthermore, the mechanisms relating calcium excretion to total serum calcium, apply only when renal function is normal, serum protein concentrations is normal, and sodium reabsorption is not greatly altered by dietary changes or drugs. Changes in previous parameters alter glomerular or tubular delivery rates reduce filtered load, or greatly alter

proximal reabsorption rates, respectively, any of which alter overall calcium excretion (**Peacock *et al.*, 1969 and Nordin et al ., 1993**).

1.1.10 Effect of hormones, minerals, nutrients on urinary calcium excretion

Parathyroid hormone (PTH) directly reduces the urinary clearance of calcium. **Eisenberg (1968)** reported that under controlled conditions a two- to three-fold reduction in urinary calcium clearance in humans can be achieved by parathyroid hormone administration. Also, it stimulates the reabsorption of calcium in the tubules and this will reduce excretion. The overall of its effect on urinary excretion of calcium is thus variable, depending upon the relative magnitude of the two effects (**Brook and Marshall, 2001; Guyton and Hall, 2001**).

Estrogen lowers urinary calcium excretion; estrogen deprivation after oophorectomy causes a rise in urine and serum calcium (**Gallagher and Wilkinson., 1973 and Amins *et al.*, 2000**). However, administration of large quantities of cortisone or other glucocorticoids increases urinary calcium excretion. Also, growth hormone leads to hypercalciuria, apparently by direct renal effect (**Bijvoet *et al.*, 1977 and Ganong, 2001**). Calcitonin, causes hypercalciuria even in hypoparathyroid person.The hypocalcaemic effect of calcitonin depends primarily on its ability to inhibit the mobilization of calcium from bone. Secretion of calcitonin is stimulated by hypercalcaemia, which at the same time suppresses secretion of PTH (**Cramer *et al. 1969*, Rasmussen and Feinblatt .,1971 and Kenneth, 1998**). Nevertheless, the physiological role of calcitonin remains uncertain, although increased concentrations of calcitonin are found in pregnant and lactating women, and so it may be more important under these conditions (**Brook and Marshall, 2001 and Guyton and Hall, 2001**). On the other hand, the effect of glucocorticoids

seems not to be a direct action on the kidney but rather an increase in bone mineral release (**Bijvoet *et al.*, 1977 and Goodschalk *et al.*, 1992**).

Increased oral intakes of inorganic phosphate leads to a reduction in urinary calcium excretion by a direct effect on reduction of renal calcium clearance and not by enhaneing deposition of calcium in bone as was believed (**Eisenberg, 1968**). Conversely, hypercalciuria is noted in severe phosphate depletion (**Clark *et al.*,1971**).A high intake of magnesium leads to increased urinary excretion of calcium. Metabolic acidosis leads to hypercalciuria; the explanation of mechanism is not clear, but it may be an indirect effect, with an increased filtered load secondary to increased bone resorption (**Bijvoet *et al.*, 1977 and Massey and Whiting., 1993**).

1.2 PHOSPHATE

1.2.1 Distribution

In contrast to calcium, phosphate is widely distributed in non-osseous tissues in both inorganic form and as a component of various structural and functional macroglobules, including phospholipids, phosphoprotein, nucleic acids, glycogen, and other intermediates of carbohydrate metabolism (**Irving *et al.*, 1964; Heaney, 1986 and Ganong, 2001**).

Eighty-five percent of body phosphate is in the mineral phase of bone, and the remainder is located in inorganic or organic form throughout the extracellular and intracellular compartments. In human serum, inorganic phosphate is present at a concentration of approximately 2 mM and exists almost entirely in ionized form as either H_2PO_4 or HPO_4. Only 12% of serum phosphate is protein-bound, and an additional small fraction is loosely complexed with calcium, magnesium, and other

cations. Intracellular free phosphate concentrations are generally comparable to those in the extracellular fluid (i.e, 1 to 2 mM), although the inside- negative electrical potential of the cell creates a significant energy requirement for translocation of phosphate into cells. This process generally is accomplished through sodium-phosphate co-transport driven by the transmembrane sodium gradient. A number of sodium-phosphate cotransport driven by the transmembrane sodium gradient. A number of sodium-phosphate cotransporters have been cloned; various cells and tissues employ different species of such transporters with distinctive regulatory characteristics (Figure 3) **(Krane, 1970; Elin *et al.*, 1971 and Guyton and Hall, 2001).**

Organic phosphate is a key component of virtually all classes of structural, informational, and effector molecules that are essential for normal genetic, developmental, and physiological processes. Phosphate is an integral constituent of nucleic acids; phospholipids; complex carbohydrates; glycolytic intermediates; structural signaling, and enzymatic phosphoproteins; and nucleotide cofactors for enzymes.

G proteins of particular importance are the high – energy phosphate ester bonds present in molecules such as adenosine triphosphate (ATP), diphosphoglycerate, and creatine phosphate that store chemical energy. Phosphate plays a particularly prominent role as the key substrate or recognition site in numerous kinase and phosphatase regulatory cascades. Cytosolic phosphate per se also directly regulates a number of crucial intracellular reactions, including those involved in glucose transport, lactate production, and synthesis of ATP **(Elin *et al.*, 1971; Heany, 1986 and Ganong, 2001).**

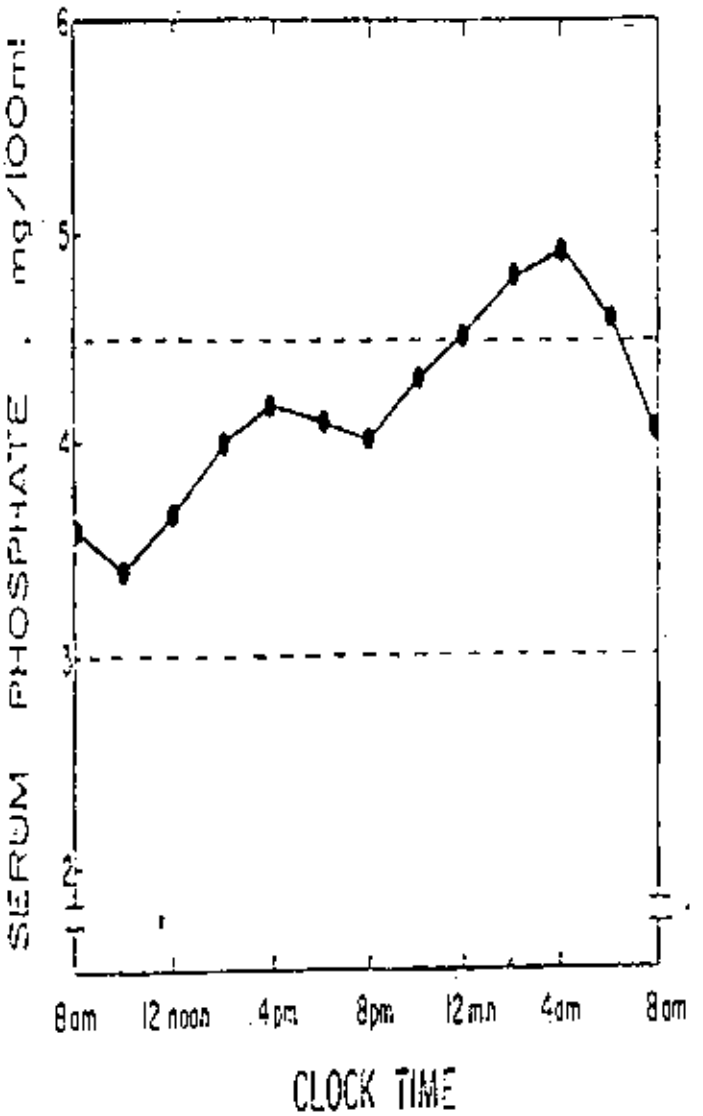


Fig. 3 The diurnal variation in serum phosphate concentration.

(Adapted from data of Jubiz et al., 1972).

In light of these diverse roles, it is not surprising that disorders of phosphate homeostasis associated with severe depletion of intracellular phosphate lead to profound and global impairment of organ function (**Elin *et al.*, 1971**). The fasting serum concentration of phosphate is modified by some factors including age and hormonal status, with higher levels during childhood and in post menopausal females (**Young and Nordin .,1967; Corvillain and Abramow., 1972; and Dick *et al.*, 2004**).

1.2.2 Absorption

The average dietary intake of phosphate, derived largely from dairy products, and cereals is 800-900 mg/day; roughly twice the estimated minimal requirement of 400 mg/day. Absorptive efficiency average about 70% but may increase up to 90% if dietary phosphate falls below 2mg/kg/day (**Krane, 1970 and Guyton and Hall, 2001**).

The mechanism of intestinal phosphate transport has not been investigated as intensely as has that of calcium it is shown that phosphate absorption occurs via an active process when phosphate concentration in the intestinal lumen is below 3 mM. However, above this concentration, transport proceeds mainly by passive diffusion (**Harrison and Harrison., 1961 and Ganong, 2001**).

The inorganic phosphate in the plasma is filtered in the glomeruli, and 85-90% of the filtered is reabsorbed. Active transport in the filtered proximal tubule accounts for most of the reabsorption, some additional phosphate (8% to 10%) is reabsorbed in the distal tubule (but not in Henle's loop) and this active transport process is powerfully inhibited by parathyroid hormone (**Ganong, 2001**).

The rats and chicks phosphate absorption is maximal in the jejunum (**Wasserman *et al.*, 1973; Walling , 1977**). Other studies of

ponies and calves implicate a more distal small intestine and/or colonic site. Similar data in humans are not available. **(Yang and Thomas., 1965)**. Inorganic phosphate is absorbed in the duodenum and small intestine by both active transport and passive diffusion. However, unlike the absorption of Ca^{2+}, the absorption of P^{---} is linearly proportionate to dietary intake. Many stimuli that increase Ca^{2+} absorption, including 1,25-dihydroxycholeciferol also increase P^{-} absorption **(Tenenhouse, 1997 and Ganong, 2001)**.

About the precise role of vitamin D on the absorption of calcium, it seems reasonable at present to postulate that vitamin D may stimulate mechanisms for phosphate absorption **(Harrison and Harrison.,1961; Corradino, 1973 and Caverzasio *et al.*, 1986)**.

While translocation of phosphate may occur passively in response to active calcium transport in the duodenum, additional evidence provides further support for the existence of the calcium-independent mechanism. **(Kowarski and Schacter ., 1973 and Tenenhouse, 1997)**.

However intestinal absorption of phosphate that is excreted in the feces in combination with nonabsorbed into the blood from the gut and later excreted in the urine **(Guyton and Hall, 2001)**.

It appears that the active principle responsible for the stimulation of phosphate is the dihydroxy metabolite 1,25- dihydroxy vitamin D. This metabolite has been shown to increase both calcium and phosphate transport in deficient rats within 3 to 6 hours of administration **(Harrison and Harrison., 1961 and Daily *et al.*, 1990)**.

Surprisingly enough, the possible influence of parathyroid hormone on phosphate absorption has received little recent attention.

Older studies utilizing in vivo administration of the hormone must now be reinterpreted in the light of the known physiologic role of parathyroid hormone in the stimulation of renal hydroxylation of 25-hydroxyl vitamin D, since previously observed effects of parathyroid hormone may have been mediated by changes in level of 1,25- $(OH)_2 - D_3$ **(Borle *et al.*, 1963)**. Others have observed no effects of parathyroid hormone in vivo on phosphate transport **(Clark and Dantzler.,1972)**. Definition of the effects, if any, of calcitonin on phosphate absorption has been elusive **(Cramer *et al.*, 1969)**.

Tanzu and Navia.,(1973) adduced indirect evidence for inhibition of absorption of phosphate in rats. Moreover, they noted a significant increase in blood levels in human volunteers after administration of porcine calcitonin compared with controls. They also found that calcitonin enhances relative absorption of both calcium and phosphorus. So, the interpretation of these studies is particularly difficult and uncertain. Many of the effects of calcitonin are mediated by a G protein-coupled cell surface receptor in the PTH/secretin receptor family **(Hirsch *et al.*, 1964)**. The mRNA encoding this receptor has been found in multiple tissues, including kidney, brain and osteoclasts. The coupling of this receptors to different G proteins results in the activation of either adenylate cyclase or phospholipase C. In some settings, this effect is cell cycle-dependent **(Aurbach and Chase., 1978 and Caverzasio *et al.*, 1986)**. Glycosylation of the receptor is important for both binding and signal transduction .Several isoforms of the calcitonin receptor have been described but the functional significance of these various isoforms is not known **(Eisenberg , 1968)**.

Phosphorus absorption from small intestine increases with intralumenal pH over the range 3.3-7.9 **(Cramer, 1965 and Oster *et al.*,**

1978), more alkaline pH's are believed to interfere with phosphate absorption through the promotion of insoluble phosphate salts (**Oster *et al.*, 1978 and Guyton and Hall, 2001**).

1.2.3 Excretion

Phosphate losses in sweat are negligible (25 mg/day), even at estimated maximum rates of sweating (**Atman and Dittmer, 1968**). Studies in fasting humans have identified an obligate daily fecal phosphate loss of 200-300 mg/day in normophataemic volunteers, but the major site of phosphate excretion and regulation is clearly the kidney. Renal tubular phosphate reabsorption occurs via a saturable active transport mechanism (**Ganong, 2001**). Although the transport sites were initially localized to the proximal tubule on the basis of micropuncture studies in animals (**Tenenhouse, 1997**).

Ample evidence now supports the existence of one or more distal tubular reabsorptive sites as well (**Staum, 1972**). Moreover, recent clinical studies provided additional evidence in humans for multiple sites for phosphate reabsorption. The phenomenon of tubular secretion has been demonstrated in some species but definite proof of such a mechanism in mammals is lacking (**Clark and Dantzler., 1972 and Boudry, 1975**). Like the intestinal mechanism, proximal tubular reabsorption is saturated at physiologic concentration (2 mM) of intralumenal phosphate, and the monovalent ion (H_2Po_4) is the predominant (**Bank, 1974; Caverzasio *et al.*, 1986; and Guyton and Hall, 2001**).

The regulation of phosphate excretion to maintain phosphate balance is accomplished primarily by PTH. Which inhibits phosphate reabsorption in the proximal tubule such that 40% or more of the filtered

phosphate can be excreted at high PTH levels and less than 5% in the absence of PTH (**Guyton and Hall, 2001**). To understand how PTH can have a role in maintaining phosphate balance when the secretion of PTH is regulated primarily by the concentration of ionized Ca^{2+} in plasma (**Clark *et al.*, 1971 and Daily *et al.*, 1990**).

However, PTH can greatly increase phosphate excretion by the kidneys, thereby playing an important role in the control of plasma phosphate concentration as well as calcium concentration (**Guyton and Hall, 2001**). Finally, the possibility of unrestrained secretion of PTH, leading to excessive bone resorption and severe hypophosphatemia, is prevented by the effects of calcium on PTH secretion and by the direct suppressive effect of 1,25- $(OH)_2$ D_3 on the synthesis of PTH and receptors (**Daily *et al.*, 1990**).

1.3 ALKALINE PHOSPHATASE

Alkaline phosphatase refers to a group of zinc containing metallo-enzymes of uncertain structure and physiological function that catalyze the hydrolysis of phosphate esters in the alkaline media. The enzyme is synthesized in bone osteoblasts, leucocytes, and cell of the bile canaliculi, proximal convoluted tubule, placenta and active mammary gland. It is to be noted that, in normal individuals, alkaline phosphatase of both bone and liver origin can be detected in the serum (**Posen, 1967 and Burke ,1978**).

Alkaline phosphatase ranges normally from 10-30 kind and kind U/100 ml (71-142 IU/L) in children and growing individuals but these figures decrease little in adult, where it ranges from 3-18 kind and kind U/100 ml (21-92 IU/L). Lower than normal alkaline phosphatase activities may be present in persons suffering from severe malnutrition, in

total body irradiation achondroplasia and scurvy **(Posen, 1967 and Somjen *et al.*, 1989)**.

The enzyme is almost undetectable in the rare disorder known as hypophosphatasia. There is a close relation between the number and activity of osteoblasts in bone and the level of alkaline phosphatase in serum **(Kaplan, 1972)**.

Thus, the enzyme will be abnormally high in Paget's disease of bone, sarcomatous alteration of Paget's disease, hyperparathyroidism, rickets, osteomalacia and osteoblastic sarcoma. Secondary bone tumours do not usually generate particularly high levels of alkaline phosphatase activity, although levels are greatest in association with those metastases that incite bone formation. In growing children the levels are also significantly raised **(Posen, 1967 and Somjen *et al.*, 1989)**.

The intestine occasionally contributes to an abnormal serum alkaline phosphatase activity. The level increases in steatorrhea and in postprandial state, particularly when a fat-enriched meal has been eaten **(Moss *et al.*, 1961 and Burke, 1978)**. Intestinal perforation releases the enzyme into the peritoneal cavity. Kidney alkaline phosphates is occasionally released in renal infarction and rarely in renal tumours **(Kaplan, 1972)**.

The placental syntrophoblastic cells contribute alkaline phosphatase to the serum throughout the third trimester of pregnancy. The level returns to normal by the third week postpartum. Of primary interest in clinical medicine is the variation of alkaline phosphatase activity seen in association with liver disease **(Posen, 1967)**.

Viral or toxin- produced hepatocellular disorders are not associated with striking elevations of the enzyme. However, even minimal obstructive conditions produce a 2-fold rise. When the alkaline phosphatase is elevated and the bilirubin concentration remains normal, "dissociation" is said to have occurred, and the presence of nonhepatic disorder or a space-occupying lesion within the liver must be suspected **(Kaplan, 1972 and Somjen *et al.*, 1989)**.

There are two theories to explain the increased enzyme levels seen in obstructive disease. The "retention theory" holds that normal serum alkaline phosphatase originates in the bone and is removed via biliary excretion. Therefore when obstruction occurs the enzyme cannot be removed normally and builds up in the serum **(Statland *et al.*, 1972 and Storm *et al.*, 1998)**.

Since surgical removal of the liver does not result in large increases in alkaline phosphatase, this theory is not well regarded **(Kaplan, 1972)**.The "regurgitation theory" suggests that the pressure of obstruction will produce excessive alkaline phosphatase production **(Statland *et al.*, 1972 and Somjen *et al.*, 1989)**.

1.4 THE OVERALL CONTROL OF CALCIUM AND PHOSPHORUS

1.4.1 Hormonal Control of Calcium and Phosphorus

1.4.1.1 Parathyroid hormone

Parathyroid hormone (PTH) is produced by the parathyroid glands. There are generally 4 small parathyroid glands (average weight, 35 mg) in human located close to thyroid gland, each parathyroid gland is a richly vascularized disk, about 3x6x2 mm, containing two distinct types

of cells; the abundant are chief cells, which contain a prominent Golgi apparatus plus endoplasmic reticulum and secretory granules that synthesize and secrete parathyroid hormone (PTH) **(Ganong, 2001)**.

The secretion of PTH is sensitively controlled by free calcium, thus, acutely lowered calcium concentration lead to an increase in PTH values within limits. In cows, the PTH secretion rate becomes maximal at a total calcium concentration of 8 mg% and minimal at 11 mg% **(Blum *et al.*, 1974 and Brook and Marshall, 2001)**. The secretion cannot be abolished, however, even at very high calcium levels **(Clark *et al.*, 1971 and Ganong, 2001)**. A decreased concentration of magnesium can also cause an increase in PTH secretion, but magnesium appears to be 2-3 times less effective than calcium **(Hebener and Potts., 1976; Brook and Marshall, 2001)**.

Changes in phosphorus concentration affect PTH secretion only secondary through changes in free calcium **(Grant *et al.*, 1990)**. The calcium receptor is also a G-protein linked. It is linked to Phospholipase C. Hypercalcaemia suppresses secretion, while hypocalcaemia stimulates secretion **(Brook and Marshall, 2001)**. Catecholamines have been found to increase of direct β-adrenergic stimulation of parathyroid gland **(Fischer *et al.*, 1973 and Dick *et al.*, 2004)**.

The secretion of parathyroid hormone appears to be mediated by the adenyl cyclose system **(Williams *et al.*, 1973 and Brook and Marshall, 2001)**. A large share of the effect of PTH on its target organs is mediated by the cyclic adenosine monophosphate (cAMP); second messenger mechanism. Within a few minutes after PTH administration, the concentration of cAMP increases in the osteocytes, osteoclasts, and other target cells. This cAMP in turn is probably responsible for

osteoclastic secretion of enzymes to cause bone reabsorption and formation of 1,25-dihydroxycholeciferol in the kidneys. There are probably other direct effects of PTH that function independently of the second messenger mechanism **(Brook and Marshall, 2001 and Guyton and Hall, 2001)**.

The parathyroid hormone markedly increases the efficiency of intestinal calcium absorption. The bulk of evidence indicates that this effect is indirect through stimulation of increased 1,25-dihydroxy vitamin D **(Wasserman and Taylor., 1976 and Guyton and Hall, 2001)**.

Studies done by **Potts *et al.*, (1974) and Wasserman and Taylor (1976)** showed that the effects of parathyroid hormone are not rapidly mediated, but require 24 hours or more to be expressed, consistent with an indirect action through changes in the rate of 1,25-dihydroxycholeciferol-dihydroxy vitamin D production. They also reported that this indirect effect of PTH on intestinal calcium absorption, even indirect may be the most important factor physiologically in regulation of intestinal calcium absorption **(Shimizu, 2000)**.

Parathyroid hormone has a calcium-retaining action on the kidney, probably because it depends on an increase in tubular reabsorption. Parathyroid hormone also increases renal tubular reabsorption of calcium at the same time that it diminishes phosphate reabsorption. Moreover, it increases the rate of reabsorption of magnesium ions and hydrogen ions while, it decreases the reabsorption of sodium, potassium and amino acid ions in much the same way that it affects phosphate. The increased calcium absorption occurs mainly in the late distal tubules, the collecting tubules, the early collecting ducts, and possibly the ascending loop of

Henle to a lesser extent. The effect of PTH on the kidney to increase calcium reabsorption, continual loss of calcium into the urine would eventually deplete both the extracellular fluid and the bones of this mineral (**Bijvoet *et al.*, 1977 and Guyton and Hall, 2001**).

The excessive urinary calcium loss seen in hypo-parathyroidism reflects loss of the distal renal tubular action of parathyroid hormone. Conversely, the hypocalcaemia of hyperparathyroidism is largely attributable to renal action of pathologically elevated level of PTH (**Bijvoet *et al.*, 1977**).

Parathyroid hormone produces acute phosphaturia in normals and in cases of parathyroidism. This effect is accompanied by a rise in urinary cyclic AMP both in vivo and in isolated tubules in vitro. The decline in phosphate concentration is caused by a strong effect of PTH to increase renal phosphate excretion, an effect that is usually great enough to override increased phosphate absorption from the bone (**Gill and Casper ., 1971 and Guyton and Hall, 2001**).

1.4.1.2 Vitamin D

Vitamin D is absorbed into the lymphatics and enters the circulation bound primarily to vitamin D, binding protection, although a fraction of vitamin D circulates bound to albumin (**Vander *et al.*, 2001**).

The human vitamin D-binding protein is an α-globulin, with a molecular mass of approximately 52 kd, the protein has a high affinity for 25 (OH)D but also binds vitamin D and 1,25- $(OH)_2D$. Vitamin D_3 and its hydroxylated derivatives are transported in the plasma bound to a globulin vitamin D-binding protein (DPB), which is also known as Gc protein (**Ganong, 2001**). Vitamin D in its active form (calcitriol: $1,25(OH)_2$ D_3) is essential for the absorption of calcium from the gut.

without which bone mineralization and growth is poor. It is also important for the maintenance of a stable concentration of Ca^{2+} in the ECF (**Shimizu *et al.*, 2000 and Guyton and Hall, 2001**).

Puschett, (1974) was able to detect a significant acute stimulation of renal phosphate reabsorption (increased), 25-hydroxyl vitamin D and 1.25 dihydroxy vitamin D, by vitamin D when given intravenously in supraphysiologic doses to thyroparathyroidectomized dogs. This effect was maximal within 1 or 2 hours and was produced most rapidly by 1.25-dihydroxycholeciferol dihydroxy vitamin D, which was also the most potent of the vitamin D metabolites in this respect . Furthermore, 1.25-$(OH)_2$ D_3. increases amino acid uptake and alters phospholipids metabolism in vitro in muscle cells **(Kumar, 1994).**

It seems possible that PTH and vitamin D metabolites are directly synergistic with respect to their effects on renal phosphate handling **(Rasmussen and Feinblatt .,1971 and Dawson-Hughes *et al.*, 1997).**

1.4.1.3 Calcitonin

Calcitonin, when administered acutely, decreases tubular resorption of calcium, However In rodents, calcitonin is known to play a role in the regulation of postprandial hypercalcemia. Studies in calcitonin knockout mice reveal a doubling of bone formation rate in the absence of hormone, accompanied by resistance to ovariectomy-induced bone loss **(Friedman and Gesek., *1995*).**And impairs osteoclast-mediated bone resorption by direct action on osteoclasts. The physiological role of calcitonin in humans, however, remains elusive. The effect of calcitonin on bone density was examined in patients with long-term hypercalcitoninemia secondary to medullary carcinoma of thyroid gland

(MCT) and in patients with subtotal thyroidectomy resulting in lack of calcitonin secretory reserve (**Ganong, 2001**).

Many of the effects of calcitonin are mediated by a G protein-coupled cell surface receptor in the PTH/secretin receptor family (**Tanzu and Navia .,1973 and Friedman and Gesek.** ***1995***). The mRNA encoding this receptor has been found in multiple tissues, including kidney, brain, and osteoclasts. The coupling of this receptor to different G proteins results in activation of either adenyl cyclase or Phospholipase C. In some settings, this effect is cell cycle-dependent (**Tanzu and Navia., 1973**).

1.4.1.4 Androgens

All androgens are steroid compounds, as shown testosterone and dihydrotestosterone, androgens can be synthesized either from cholesterol or from acetyle coenzyme A. Androgens are necessary for bone strength in males. They may decrease with ageing, but the role of testosterone treatment is not clear. They are also known as "anabolic steroids" and are abused by some athletes, may increase bone formation in females, menopause contributing to development of osteoporosis (**Guyton and Hall,2001**). Clinical observation have suggested that androgens play a vital role in bone (**Nordin *et al.*, 1966 and Ganong, 2001**).

Bernard , (1963), received data derived from human treated with three androgens (testosterone proprionate, 2-methyldihydro-testosterone propionate and fluoxymesterone). They noticed that androgens lower the serum calcium level. This could not be ascribed to renal wasting of calcium, since calcium clearance did not increase. Thus, the fall of serum calcium level probably reflected increased calcium deposition or decreased calcium release from bone reservoirs.

Eisenberg et al, (1964) has shown that androgens does not accelerate deposition of bone-seeking mineral, as measured by nonradioactive strontium, so that the well known "anabolic" effect of androgens on bone is in fact "anticatabolic". Therefore, it is likely that the fall in serum calcium levels during androgen treatment reflects a decrease in osteolysis. The similar significant fall of serum phosphate level in androgen treated individuals may be explained by the same mechanism. The lowering of serum phosphate levels by androgen treatment of osteoporosis was noted by **Albright and Reifenstein (1993).**

Bernard ,(1963) came to a conclusion that androgen treatment causes a fall in serum calcium and phosphate levels without significant change in calcium clearance, or in tubular reabsorption of calcium or phosphate. These observations are interpreted to mean that androgen decreases osteolysis, without significant changes in any measured renal function. Also he adviced for the use of androgens in patients suffering from disseminated metastasis in bone to decrease osteoporosis. Recently, **Kasperk et al., (1990)** reported a direct effect of androgens on murine and human bone cells to stimulate bone cell proliferation and differentiation. To test whether this effect of androgenic steroid might be mediated by growth factors, they measured relative concentration of insulin-like growth factor-I and -II (IGF-I and IG F-II) and transforming growth factor-beta (TGF beta) in the condition medium from androgen-treated murine calvarial cell cultures. Only the concentration of TGF beta was increased; consistent with the increased secretion of TGF beta in the mouse calvarial cell system. They observed an increased expression of TGF beta mRNA in a normal human osteoblastic cell system. They also determined whether androgens alter the response to growth factor. They found that dihydrotestosterone (DHT) treatment enhanced the mitogenic

effects of fibroblast growth factor (FGF) and IGF-II but not those of IGF-I. The enhanced effect of FGF and IGF-II after DHT pretreatment was not affected by addition of TGF beta-blocking antibodies or by changing the culture medium. This indicated that in addition to increased release of TGF beta, another mechanism might be involved in the action of DHT on human and murine bone cells. Thus, they investigated the binding of human IGF-II to human osteoblastic cells and observed an increase in IGF-II binding after DHT treatment. Their results are consistent with a mechanism of action of androgens on bone cells that involves the induction of TGF beta and, in addition , may sensitize the cells to show an enhanced response to FGF and IGF-II (**Kasperk *et al.*, 1989 and Kasperk *et al.*, 1990**).

Anderson *et al* (1997), therefore conclude that testosterone is a promising treatment for men with idiopathic osteoporosis, acting to suppress bone resorption by a mechanism that may involve estrogen. Androgens can increase bone formation in vivo, and testosterone increases the total quantity of bone matrix and causes calcium retention. The increase in bone matrix is believed to result from the general protein anabolic function of testosterone plus deposition of calcium salts in response to the increased protein (**Falahai *et al.*, 2000 and Guyton and Hall, 2001**).

Anderson *et al.*, (1997) conducted an open study to investigate the efficacy and mode of action of testosterone therapy in eugonadal men with osteoporotic vertebral crush fracture. Twenty-one men, aged 34-73 (mean 58), were treated with intramuscular testosterone esters (Sustanon 250) every 2 weeks for 6 months. Bone mineral density, bone metabolic density (BMD) measurement by dual-energy X-ray absorptiometry was performed at baseline and 6 months. They also measured biochemical

markers of bone turnover, testosterone, estradiol, sex hormone binding globulin (SHBG), and gonadotrophins baseline and after 3 and 6 months of treatment. They therefore concluded that testosterone is a promising treatment for men with idiopathic osteoporosis, action to suppress bone resorption by a mechanism that may involve estrogen.

The sex steroid androgens and estrogens are major regulators of bone metabolism. However, whether these hormones act on bone cells through direct or indirect mechanisms has remained unclear. A nuclear binding assay recently used to demonstrate estrogen receptors in bone **(Eriksen *et al.*, 1988).** Androgen receptor gene expression in osteoblasts was confirmed by RNA blot analysis. Relative concentrations of androgen and estrogen receptors were compared by measuring specific nuclear estrogen binding, nuclear binding of $(H)^3$ and they conclude that both androgens and estrogens act directly on human bone cells through their respective receptor-mediated mechanisms **(Douglas *et al.*, 1989 and Falahai *et al.*, 2000).**

1.4.1.5 Estrogens

Are the principal circulating sex steroids in females. They regulate the rates of bone formation and bone resorption, decrease after menopause, so, contributing to development of osteoporosis. The estrogen cause increased osteoblastic activity in the bone. Therefore, at puberty, when the female enters her reproduction years, her growth in height becomes rapid for several years. However, estrogens have another potent effect on skeletal growth. They cause uniting of the epiphyses on skeletal with the shafts of the long bones. This effect is much stronger in the female than in the similar effect of testosterone in the male. As a result, growth of the female usually ceases several years earlier than growth of the male. The female eunuch who is devoid of estrogen

production usually grows several inches taller than the normal mature female because her epiphyses do not unite at normal early time (**Guyton and Hall, 2001**).

Estrogen decreases urinary calcium excretion. Estrogen deprivation after oophorectomy causes a rise in urine and serum calcium. It seems that estrogen effect is indirect. The hormone antagonizes parathyroid hormone action. Another aspect of the hypocalciuric effect of estrogen is the well-known antagonism by estrogen of growth-hormone action, since the latter is hypercalciuric (**Gallagher and Wilkinson .,1973 and Lips *et al.*, 1989**). Serum phosphate rises significantly after the menopause or oophorectomy (**Dick *et al.*, 2004**) which suggests that estrogens may decrease phosphate reabsorption from the kidney. Since the estrogens may stimulate PTH secretion, (**Riggs, 1973 and Ganong, 2001**), aportion of the estrogen effect on tubular phosphate reabsorption may also results from direct antagonism between estrogen and growth hormone action in the kidney (**Guyton and Hall 2001**).

Research has lead to the general agreement among physicians and research is that the progression of bone loss can be halved in postmenopausal women with estrogen replacement therapy (ERT) (**Douglas *et al.*, 1989**). A lack of estrogen in postmenopausal woman prevent the absorptic and utilization of calcium and is the single most important factor in the development of osteoporosis in older women. ERT can reduce the risk of osteoporosis if taken within three to five years after menopause (**Steven and Whiting., 2004**).

Estrogen and progestrone supplements have also been proven to reduce the bone loss associated with osteoporosis. Women's bones

slowly begin to lose minerals and become less dense even before menopause. After menopause, however, the pace accelerates rapidly for five to ten years. Estrogen inhibits bone reabsorption and progestrone stimulates bone formation. Unless a woman is taking these hormones, she has about a one-in-four chance of developing serious osteoporosis. Although, oestrogen is the therapy of choice for prevention and treatment of osteoporosis although supplemental calcium, diet, and exercise are also beneficial. They don't seem to be as effective as estrogen **(Dick *et al.*, 2004 and Steven and Whiting., 2004)**.

High postmenopausal endogenous estrogen concentration are an important determinant of preservation of bone mass and reduced fracture in elderly women. Calcium supplementation can also reduce bone loss in these patients suggesting an interaction between estrogen deficiency and calcium balance. Potential mechanisms of estrogen on calcium transport include direct effects on the bone, the kidney and the bowel. Previous studies have demonstrated effects of estrogen on renal phosphate handling, researchers have used a cross sectional population-based analysis of biochemical data obtained from ambulant elderly women to determine the association of endogenous estradiol with urine calcium and phosphorus excretion. The estradiol and free estradiol (FE) effect on renal calcium excretion remained significant after adjusting for calcium filtered at the glomerulus and serum PTH. A high FE was associated with a reduced renal phosphate. The effect remained significant after adjustment for serum PTH. The size of the effect of the free estradiol was of the same order of magnitude as the effect of PTH on reducing renal calcium excretion and increasing renal phosphate excretion **(Dick *et al.*, 2004)**. Adult women have less mass than adult men, and after menopause they initially lose bone more rapidly than men of comparable

age. Consequently, they are more prone to development of serious osteoporosis. The cause of bone loss after menopause is primarily estrogen deficiency and estrogen treatment arrests the progress of the disease. Estrogen inhibits secretion of cytokines such as interleukin (IL-1 and IL- 6) and tumor necrosis factor (TNF) and these cytokines fasten the development of osteoclasts. Estrogen also stimulates production of transforming growth factor TGF-ß, and this cytokine increases apoptosis of osteoclasts. There are estrogen receptors on osteoblasts. Large doses of estrogens may increase the incidence of myocardial infarction and stroke, but small doses that are effective in slowing bone loss actually protect against cardiovascular disease. Estrogen alone increases the incidence of endometrial cancer, but it appears that this can be avoided if the estrogen is given with a progestine on the other hand. Prolonged treatment with estrogen may increase the incidence of breast cancer, therefore the decision to treat a postmenopausal women with estrogen depends on a careful weighing of the risk benefit ratio (**Ganong, 2001**).

1.4.1.6 Progesterone Hormone

The human skeleton accumulates bone up to approximately age 30, after which bone is gradually lost. Although estrogen replacement therapy prevents postmenopausal bone loss, it is not certain that estrogen deficiency alone is responsible for the decrease in bone mass. Progesterone deficiency could also be a factor, and progesterone replacement therapy has been shown to prevent postmenopausal bone loss associated with ovarian dysfunction. Progesterone plays an important role in regulating bone formation, and suggests directions for future studies in predicting the success or failure of implant therapy based on the number and kinds of osteoprogenitor cells present (**Lee, 1990 and Heersche *et al.*, 1998**).

Local and systemic insulin-like growth factors (IGFs) may be involved in the regulation of bone formation by sex hormones. The following study describes the in short term, estrogen and in vivo effects of estradiol, progesterone, or both on IGF-mRNA abundance in bone, serum IGF-I level, and on bone formation. Rates were Sham-operative (SHAM) or ovariectomized (OVX) at 12 weeks of age and used a week later in three experiments. First, OVX rats were treated with vehicle, estradiol, and/or medroxyprogesterone (MPA) for 3 weeks, and bone formation was assessed in the tibial metaphysis. Second, OVX rats were treated with an injection of vehicle, estradiol, and/or progesterone. 24 hours later, levels of IGF-I mRNA in the femur were analyzed. The mineralized surface, mineral opposition rate, and bone formation rate (BFR) were higher in OVX than in SHAM rates. The BFR was decreased in estrogen-treated but increased in MPA-treated rats compared with vehicle-treated OVX rats. Circulating levels of IGF-I were higher in OVX than in SHAM rats but were not affected by sex hormones in a 3-week experiment, whereas these levels were not different among groups in a 24-h experiment. The abundance of IGF-I mRNA was higher in OVX than in SHAM rats. They concluded that in the short term, estrogen lowers and progesterone raises bone IGF-I mRNA and these changes are followed by coordinated changes in bone formation rate. Esteopenic OVX rats are mediated through a direct activity on bone **(Barengolts *et al.*, 1996)**.

Osteoporosis, however, begins at about age 35 years when progesterone begins to decline despite continued good esterogen level. This progesterone deficiency is the major cause of the early appearance of the process. At menopause, estrogen levels decline markedly. This abrupt decline in estradiol results in an acceleration of osteoclast-

mediated bone resorption. Also, there is decrease of progesterone receptors, thus decreasing new bone formation years around menopause. It is not unusual to observe industrialized decrease of bone metabolic density (BMD) in the range of 3-4% per year. compared to 1-1.5% per postmenopausal life. Moreover, progesterone stimulates osteoblast-mediated formation (**Marcus. 1991: and John and Lee, 2006**).

1.4.1.7 Thyroxine

In children. hyperthyroidism is associated with increased skeletal growth and hypothyroidism result in decreased growth (**Greenspan and Greenspan., 1999**).

Thyroid hormones are crucial for cartilage growth and differentiation and enhance the response to growth hormone. They increase bone turnover (**Engler *et al.*, 1999**). although their effects on bone formation are less clear, increased resorption as result of hyperthyroidism may result in a coupled increase in bone formation, but thyroid hormone may also directly stimulate bone cell replication (**Williams *et al.*, 1973 and Merton, 1999**).

Previous work has shown that T_3 increases the production of the bone growth factor, insulin-like growth factor-I (IGF-I), in osteoblastic cells, and that it promotes the production of the osteoclastogenic cytokine; interleukin-6. The experiments carried out during prior work evaluated models that could be used to study the roles of these local factors in the anabolic and resorptive effects of T_3 on bone, and molecular mechanisms by which T_3 stimulated their production. The early findings. using antibodies to the IGF-I and IL-6 receptors, and antisense oligonucleotides to the IGF-I receptor, support a role for these factors in the thyroid hormone effects. Recent studies. at the level of gene

expression. show T_3 augmentation of IL-6 production in human and in rodent osteosarcoma cells; a lack of effect of T_3 on IL-I receptor expression; and T_3 potentiation of parathyroid hormone hormone-induced IL-6 mRNA production (**Greenspan and Greenspan., 1999**).

1.4.1.8 Insulin

Normal skeletal growth depends on an adequate amount of insulin (**Rosen and Donahue., 1998**). Excess insulin production by the fetuses of mothers with uncontrolled diabetes results in excessive growth of the skeleton and other tissue and under treated diabetes mellitus impairs skeletal growth and mineralization. In vitro, insulin at physiologic concentration selectively stimulates osteoblastic collagen synthesis by a pretranslational mechanism. Insulin can mimic the effects of IGF-I but only at supraphysiologic levels. Insulin does not appear to affect bone resorption (**Williams *et al* ., 1973 and Rosen and Donahue., 1998**).

Insulin increases bone formation and there is significant bone loss in untreated diabetes (**Brook and Marshall., 2001 and Ganong, 2001**).

1.4.1.9 Growth Hormone

Growth hormone promotes the reabsorption of Ca^{2+}, phosphate and Na^{+} by the kidney and growth hormone promote bone growth by stimulating osteoblastic synthesis of collagen (**Ganong, 2001**).

Growth hormone causes an acute fall in urinary phosphate excretion. an elevation of fasting serum phosphate and an increase in the renal phosphate threshold (**Corvillain and Abramow ., 1962 and Guyton and Hall, 2001**).

Growth hormone increases calcium excretion in the urine, but it also increases its intestinal absorption and this effect may be greater than

the effect on excretion, with a resultant positive calcium balance (Ganong, 2001).

1.4.2 Other factors affecting calcium and phosphorus homeostasis

1.4.2.1 Prostaglandins

Prostaglandins are potent regulators of bone cell metabolism and are synthesized by many cell types in the skeleton (**Pilbeam *et al.*, 1996**). Prostaglandin production in bone is regulated by the effects of local and systemic hormones and mechanical forces on the inducible cyclooxygenase (COX-2). Increased prostaglandin production may contribute to the increase in bone resorption with immobilization, the increase in bone formation with impact loading, and the changes after estrogen withdrawal. Many of the hormones, cytokines and growth factors that stimulate bone resorption also increase prostaglandin production. Prostaglandins have biphasic effects on bone formation. Stimulation of bone formation is seen in vivo and inhibition of collagen synthesis occurs in osteoblast cultures. Bone cells produce PGE_2, $PGF_{2\alpha}$, prostacyclin, and lipoxygenase products (e.g. leukotriene B_4), which may also stimulate bone resorption (**Williams *et al.*, 1973**).

1.4.2.2 Cytokines

Interleukin IL-1α, IL-1, TNA-α and tumor necrosis factor TNF-β (TNF-β) are potent stimulators of bone resorption and inhibitors of bone formation and may mediate bone loss after estrogen withdrawal (**Lorenzo, 1991 and Pacifici, 1998**).

Interleukin IL-6 increases osteoclastogenesis in cell cultures and may mediate some of the resorbing activity of PTH (**Lorenzo, 1991**).

IL-6 is produced by osteoblasts and its production is stimulated by PTH, PGE_2 and other factors that increase resorption. IL-11, another member of the IL-6 cytokine family, colony-stimulating factor are probably important in the early stage of osteoclast formation. IL-4 and IL-3 inhibit resorption and prostaglandin synthesis in bone cells **(Pacifici, 1998)**, and leukemia inhibitory factor has biphasic effects on bone formation Whereas IL-18 is inhibitory through its ability to increase production of granulocyte-macrophage colony-stimulating factor **(Malava *et al.*, 1995)**.

Interleukin IL-7 stimulates β lymphoiesis, which may be involved in osteoclastogenesis **(Miyaura *et al.*, 1997)**. IL-10 is an inhibitor of osteoclastogenesis and bone resorption, IL-15 and IL-17 stimulate it **(Owens and Chambers., 1995 and Ganong, 2001)**.

Interferon inhibits resorption by inhibiting osteoclast responses to PANKL **(Takayanagi *et al.*, 2000)**.

In addition to direct effects, responses to cytokines can be blocked by inhibitors, such as the IL-1 receptor antagonist and the soluble TNF receptor, or they can be enhanced by activators such as the soluble IL-6 receptors **(Lorenzo, 1991)**.

1.4.2.3 Insulin-like Growth Factors

IGFs increase bone cell replication, matrix synthesis and bone formation **(Rosen and Donahue.,1998)**. Both IGF-I and IGF-II are synthesized by bone cells and stored in bone matrix. More IGF-II is stored in human bone, but IGF-I is a more potent stimulator of osteoblasts. Binding of IGF-I and IGF-II to matrix may be mediated by specific IGF-binding proteins. Five of the six known binding proteins have been identified in bone, and these both inhibit and enhance IGF responses. Because PTH and PGE_2 increase and glucocorticoids decreased skeletal IGF-I

synthesis, IGFs may mediate the effects of these hormones on bone growth. IGF-I and its binding proteins may also stimulate osteoclast formation (**Hill *et al.*, 1995**).

1.4.2.4 Magnesium

Is responsible for many biochemical processes within the bone. Magnesium is essential for the conversion of vitamin D to its biologically active form. More than one-half of the body's magnesium is stored in the bones; most of the rest resides within the cells, with less than 1% located in the extracellular fluid (**Walser ,1967; Morgan., 1985 and Guyton and Hall, 2001**).

Magnesium infusion transiently reduces urinary phosphate in dog's body, despite an increase in serum phosphate . This action may be mediated or dependent upon parathyroid hormone. Since it is not observed in parathyroidectomized animals (**Massry *et al.*, 1970,Walling 1977 , and Ganong, 2001**).

In normal adult the maintenance of Mg^{2+} balance involves the reabsorption of 90-99% of the filtered Mg^{2+}.The mechanisms that regulate magnesium excretion are not well understood, but the following disturbances lead to increased magnesium extraction (1) Increased extracellular fluid magnesium concentration; (2) Extracellular volume expansion; and (3) Increased extracellular fluid calcium concentration (**Guyton and Hall, 2001**).

1.4.2.5 Potassium

There have been several reports of hypophosphatemia, hyperphosphaturia and reduced phosphate reabsorption accompanying severe hypokalemia in humans (**Oster *et al.*, 1978 and Ganong, 2001**).

The high concentration of K^+ in intracellular is generated by the Na^+/K^+ ATPase. which is present in the plasma membrane of all cells. including red cells which are highly permeable to K^+. Under normal conditions a steady state develops in which the quantity of K^+ pumped into cells by the Na^+/K^+ ATPase is equal to total passively diffusing out of cells **(Guyton and Hall, 2001)**.

With no physiological regulation of K^+ input, K^+ balance must be achieved by changing K^+ output to match K^+ input as indicated above. K^+ output is regulated primarily by changing amount of K^+ excreted in urine. Potassium is reabsorbed in segments 1 and 2 of the proximal tubule, secreted in segments 3 of the proximal tubule and the thin, descending limb of the loop of Henle, reabsorbed in the thick ascending limb of the loop of Henle and both reabsorbed and secreted in the distal nephrone **(Malvin and Lotspeich ., 1956 and Guyton and Hall, 2001)**.

1.4.2.6 Hydrogen ion

Regulation of hydrogen ion balance is similar in some ways to the regulation of other ions in the body. For instance, to achieve homeostasis, there must be a balance between the intake or production of hydrogen ions and the net removal of hydrogen ions from the body. The kidney plays a key role in regulating hydrogen ion removal and lungs that are essential in maintaining normal hydrogen ion concentration in both the extracellular and the intracellular fluid **(Cramer, 1965 and Guyton and Hall, 2001)**.

The administration of sodium bicarbonate to humans, dogs or rats produces phosphaturia despite a fall in serum phosphate which is indicative of a decrease in tubular phosphate reabsorption. This effect is due to both direct effect, via expansion of extracellular fluid volume and

indirect one via parathyroid stimulation consequent to the fall in the level of ionized calcium induced by alkalosis **(Malvin and Lotspeich ., 1956, Cramer, 1965 and Guyton and Hall, 2001).**

Some studies indicated that acidosis leads to inhibition of tubular phosphate reabsorption **(Guyton and Hall, 2001).** In acute respiratory acidosis, there is decrease in tubular reabsorption of phosphate **(Vander *et al.*, 2001).**

CHAPTER TWO

MATERIAL AND METHODS

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

This experimental work was carried out at Fazan diagnostic laboratory and the Sebha central laboratory, where 255 person of either sex participated in this study. Full history was taken from every one were all apparently healthy. This included past history of taking drugs, previous illness or surgical operation, any complaint, abnormal habits, medications or cigarette smoking. None of the adult females was pregnant, or complaining of any menstrual, gynaecological nor obstetrical disturbance.

Persons divided chronologically into 4 groups i.e., A, B, C and D as Table bellow

Group	Symbol	Number	Age (year)	Subgroups number and sex
First (1) Children	A	31	Less than 15	Male (A_1) (n)=15) Female (A_2) (n=16)
Second (2) Middle age	B	127	20 – 35	Male (B_1) (n=29) Female (B_2) (n=98)
Third (3) Late age	C	65	35 – 55	Male (C_1) (n=16) Female (C_2) (n=49)
Fourth (4) Elder	D	32	Above 55 (55 – 72)	Male (D_1) (n=14) Female (D_2) (n=18)

2.2 Sampling of blood

Venous blood sample take, from each fellow. It collected, early in the morning in centrifuge tube, containing no anticoagulant. Each tube was, then centrifuged for 4-6 minutes in order to separate serum from cells. Fine pipettes to be analyzed separated sera. To avoid hemolysis of cells, All precautions were taken. In each sample, were estimated the following parameters.

1) Calcium
2) Phosphorus
3) Alkaline phosphatase
4) pH
5) Some hormones, including
    a. Thyroid stimulating hormone (TSH)
    b. Tri-iodothyramine (T_3)
    c. Tetraiodothyronine (T_4)
    d. Parathyroid hormone (parathormone-PTH)
    e. Testosterone
    f. Estradiol
    g. Progesterone

It is to be noted that blood samples were taken during mid-luteal phase in adult fertile females.

2.3 Sampling of urine

Morning urine samples were taken from each individual for estimation of

1) Calcium
2) Phosphorus
3) Ph

2.4 Procedures

2.4.1 Estimation of calcium by colorimetric method

2.4.1.1 Reaction Principle

Calcium ions form a violet complex with O-cresolphthalein complexone in an alkaline medium **(Barneett and Wasserman., 1973)**.

Reagents

Contents	**Initial concentration of solution**
Standard calcium	2.5 mmol/l (10 mg/l)
Buffer 2-amino-2-methylpropanol-1-01	3.5 mol/1, PH 10.7
Chromogen	
O-cresolphthalein complexone	0.16 mmol/l
8-hydroxyguinoline	6.89 mmol/l
Hydrochloric acid	60 mmol/l
EDTA	150 mmol/l

2.4.1.2 Procedure

Wavelength	Hg 578 nm (550-590 nm)
Spectrophotometer	570 nm
Cuvette	1 cm light path
Measurement	only on blank required per series
Temperature	20-25°C/37°C

	Reagent blank	**Standard**	**Sample**
Sample	-	-	25 µl
Distilled water	25 µl	-	-
Standard	-	25 µl	-
Working reagent	1.0 µl	1.0 µl	1.0 µl

Mix. read absorbance of the sample (A sample) and standard (A standard) against the reagent blank after 5 to 50 minutes (**Barnett and Wasserman., 1973**).

2.4.2 Estimation of phosphorus by colorimetric method

2.4.2.1 Method

Phosphate reacts with molybdate in strong acidic medium to form a complex. The absorbance of this complex in the near UV is directly proportional to the phosphate concentration (**Daly and Ertingshausen., 1972 and Gamst and Try., 1980**).

Reaction Principle (Simplified)

7HPO4 + 12 (MO7 O24)6 + 51 H+ → 7[P(MO12 O40)]3- + 36 H2O

Reagent

Reagent	2x100 ml Reagent Ammoniumheptamolybdate Sulfuric acid (PH<1.0)	 0.3 mmol/l 0.5 mol/l
	Detergent Activators and stabilizers	1%
Standard	1x5 ml standard Phosphorus	10 mg/dl Or 3.2 mmol/l

2.4.2.2 Procedure

Wavelength 340 nm. Hg 334 m

Optical path 1 cm

Temperature 20-25°C

Measurement against reagent blank: one reagent blank per series is required.

Pipetting Scheme

Pipette into cuvettes	Reagent blank	Sample or STD
Sample/STD	---	10 µl
RGT	1000 µl	1000 µl

Mix. incubate at least for 1 minute at room temperature. Measure the absorbance of the sample and the (standard) STD against the reagent blank within 60 minutes (A).

Calculation of the phosphorus concentration

$$C=3.2x\frac{A\,Sample}{A\,STD}(mg/dl)$$

$$C=3.2x\frac{A\,Sample}{A\,STD}(mmol/l$$

Normal values Inorganic phosphorus

Adults	2.5-5.0 mg/dl	0.80-1.62 mmol/l
Children	4.0-7.0 mg/dl	1.30-2.26 mmol/l

2.4.3 Estimation of alkaline phosphate (ALP) according to Epstein et al, (1986) and Dufour et al, (2000)

Determination of alkaline phosphatase in serum or plasma conforming to the recommendations of Deutsche gesellschaff for clinical chemistry.

$$\text{P - nitrophenyl phosphate } H_2O \xrightarrow{ALP} \text{4 - nitrophenol + phosphate}$$

Reagent

R1 DEA	Diethanolamine buffer	1 mol/l
	pH 9.8 (37°C)	
	MgCl2	0.5 mmol/l
R2 substrate	Sodium p-nitrophenyl	
	Phosphate (PNPP)	10mmol/l

2.4.3.1 Procedure

Wavelength	405 nm (Hg-400-Hg 420)
Cuvette	1 cm light path
Temperature	25°, 30°, 37°C
Measurement	against air or distilled water

The working solution must be brought at the choosed temperature for the analysis before use.

Pipette directly	1000 µl
Working solution	
Equilibrate (25, 30, 37°C)	
Sample	20 µl

Mix and wait for 1 minute. Read the initial absorbance and start timer immediately. Read again at constant intervals for 3 minutes.

Calculation

Calculate the average value of variation of absorbance per minute (ΔOD/mn) and obtain the enzymatic activity value of the sample, using the following formula

405 nm ALP (U/L) = ΔOD/nm x 2750

410 nm ALP (U/L) = ΔOD/nm x 2910

Reference Values

	25°C	30°C	37°C
Children	Up to 400 U/l	Up to 500 U/l	Up to 650 U/l
Adults	40-190 U/l	50-230 U/l	70-300 U/l

2.4.4 Estimation of Total Tri-iodothyronine according to Young et al (1975) and Chopra et al (1977) [ELISA technique]

2.4.4.1 Principle of the method

This micro plate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations in a direct determination of total T3. The adjuvant of nonspecific antiserum and the discovery of blocking agents to the T3 binding serum proteins has enabled the development of procedurally simple immunoassays. Micro-plates are coated with T3 antibodies on mixing immobilized antibody.

Enzyme T3 conjugate and a serum containing the native T3 antigen a competition reaction results between the native T3 and the enzyme T3 conjugate for a limited number of insolubilised binding sites. After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The activity of the enzyme present on the surface of the well is quantitated by reaction with a

suitable substrate to produce colour. The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum reference of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained from comparison to the dose response curve, an unknown specimens activity can be correlated with total triiodothyronine concentration (Chopra et al., 1977 and Young et al., 1975).

2.4.4.2 Procedure

Allow all reagents standards and controls to reach room temperature (20-30°C).

Format the micro-plate wells for each standard and patient specimen to be assayed in duplicate.

Essay schematic schedule

Reagents	Standards	Samples
Mark the wells		
Standards and samples	50 µl	50 µl
T3 Enzyme Conjugate	100 µl	100 µl

Swirl gently to mix for 20-30 seconds and cover.

Incubate for 60 minutes at room temperature.

Discard the contents of the micro-well plate by decanting or aspiration. If decanting, blot plate dry with absorbent paper.

Wash as per washing instructions		
TMB Substrate	100 µl	100 µl

Incubate for 15 minutes at room temperature		
Stop Solution	100 µl	100 µl

Swirl gently to mix for 15-20 seconds.

Measure absorbance at 450 nm within 30 minutes of addition of stop solution.

Refer to preparation of reagents. Always add in the same sequence as the TMB substrate.

2.4.5 Estimation of (T4) Total Thyroxin [ELISA technique]

2.4.5.1 Principle of the Method

Monoclonal antibodies specific to T4 are immobilized on micro well plates. T4 is conjugated to horseradish peroxides (HRP). In the assay, T4 is released from its binding proteins by ANS (8-anilinonaphthalene 1 sulphonic acid) present in the assay buffer.

Total T4 in the specimen competes with HRP. Labeled T4 for binding to the immobilized monoclonal antibody. After washing step enzyme substrate is added. The amount of total T4 in the sample is inversely proportional to enzyme activity. The reaction is terminated by adding a stop solution. Absorbance is measured on a micro-plate reader (Cavalieri and Rappaport ., 1977 and Liewendahl. 1990).

2.4.5.2 Procedure

1) Protect the plates from draught, strong light or direct sunlight during the test procedure. T4 assay buffer contains a light sensitive reagent ANS and should be protected from light. The incubation of sample and conjugate should be performed in the dark.

2) Careful aspiration of the washing solution is essential for good assay precision.

3) Since timing of the incubation steps is important to the performance of the assay pipette, the samples and the conjugate without

interruption pipetting of the standards and samples should not exceed 10 minutes to avoid assay drift. If more than one plate is used in the same run it is recommended to include a standard curve on each plate.

4) Adding the TMB substrate solution starts a kinetic reaction that is terminated by dispensing the stop solution. Keep the incubation times for each well the same by adding reagents at timed intervals.

5) Protect from light, absorbance values are stable for 60 minutes.

6) Plate readers measure absorbance vertically. Do not touch the bottoms of the wells.

7) It is recommended to distribute samples and standards in duplicate.

2.4.5.3 Assay Schematic

1) Allow all reagents to reach room temperature before use.

2) Dilute the wash solution.

3) Mark the wells to be used on the plate.

4) Dilute the required quantity of enzyme conjugate (1 : 200) with T4 Assay Buffer.

Reagents	Standards	Samples
Standards and Samples	50 µl	50 µl
Diluted enzyme conjugate	100 µl	100 µl

Cover and shake the plate gently for a few seconds to mix contents of the wells.

Incubate for 60 minutes at room temperature in the dark.

Wash 3 times with 300 µl/well of diluted washing solution carefully

TMB substrate solution (Timed intervals)	100 µl	100 µl

aspirating off the remaining liquid.

Cover plate and incubate for 15 minutes at room temperature in the dark.

Stopping solution	100 µl	100 µl

Cover and shake the plate gently for a few seconds to mix the contents of the wells.

Measure absorbance at 450 nm.

The stop solution should be distributed in the same sequence as the TMB substrate.

2.4.6 Estimation of Thyroid Stimulating Hormone (TSH) according to Jane (1987) (ELISA technique)

2.4.6.1 Principle of Method

A monoclonal antibody specific to the human TSH molecule is immobilized on micro-well plates and other monoclonal antibodies to the TSH molecule are conjugated with biotin.

TSH from the sample is bound to the plates and biotin conjugate is added. After a washing step streptavidin HRP conjugate is added. After a second washing step. substrate is added. The enzymatic reaction is proportional to the amount of TSH in the sample.

The reaction is terminated by the addition of stop solution. Absorbance is measured on a micro-plate reader.

2.4.6.2 Procedure

As previously mentioned in estimation of T4 in page (66, 67) except for the following steps

Protect from light absorbance values are stable for 30 minutes.

Reagents	Standards	Samples
Biotin conjugate	100 µl	100 µl
Standards and samples	50 µl	50 µl

Cover and shake for a few seconds to mix contents of the wells.

Incubate for 60 minutes at room temperature if possible on a plate shaker (approximately 2000 rpm).

Aspirate and wash wells 5 times with 300 µl/well of diluted wash solution.

Streptavidin HRP Conjugate (diluted)	100 µl	100 µl

Cover and incubate for 60 minutes at room temperature if possible on a plate shaker (approximately 2000 rpm).

Wash as above.

TMB substrate solution (at timed intervals)	100 µl	100 µl

Cover and incubate for 20 minutes at room temperature.

Stop solution (at timed intervals as per TMB substrate above)	100 µl	100 µl

Shake gently to mix.

Measure absorbance at 450-620 nm using a plate or strip reader within 30 minutes of adding stop solution.

Reference values for TSH = 0.5-5.0 µ/ml.

2.4.7 Estimation of testosterone according to Rajkowski *et al.*, (1977) and Joshi ,(1979) [ELISA technique]

2.4.7.1 Principle

Testosterone (antigen) in the sample competes with horseradish peroxidase labeled testosterone (enzyme labeled antigen) for a limited number of anti-testosterone (antibody) binding sites on microplates.

After incubation, the unbound antigen is removed by washing. The enzyme substrate (H_2O_2) and the chromogen (TMB) are added.

After incubation, the enzyme reaction is stopped and the absorbance is read in a microplate reader. Testosterone concentration in the sample is calculated based on a series of standards.

The colour intensity is inversely proportion to the testosterone concentration in the sample.

2.4.7.2 Procedure

1) At least one hour before use. bring all reagents and samples to room temperature, mixing them carefully on vortex.

2) Do not mix reagents from different lots.

3) Standards and samples should be tested in duplicate.It is recommended to prepare two wells for each of the five points on the standard curve (So-S4) and for each sample, plus one well for blank.

4) Dispensing and incubation times must be the same for all wells in the same analysis.

5) Avoid long interruptions between each step of the assay procedure.

6) Eliminate excess wash solution from the microplate after washing by blotting it gently on an absorbent paper pad.

7) Read the plate with an Elisa automatic reader capable of reading the absorbance of samples and standards at 450 nm.

8) The "blanking" of the instrument should be carried out in the blank reagent well.

Assay Schematic

Reagents	Blank	Standards	Sample
Standard (So-S4)	-	25 µl	-
Sample	-	-	25 µl
Enzyme conjugate	-	100 µl	100 µl

Mix well and cover strips with adhesive film.

Incubate for 60 minutes at 37°C.

Peel off adhesive film and aspirate the reaction solution from all wells.

Wash 300 ml/well of distilled or deionised water. Repeat wash carefully aspirating off the remaining liquid.

TMB substrate	100 µl	100 µl	100 µl

Cover strips with a new adhesive film.

Incubate for 15 minutes at room temperature (20-25°C), in the dark.

Stop solution	100 µl	100 µl	100 µl

Read the absorbance of each well at 450 nm against the blank within 30 minutes.

2.4.8 Estimation of 17-β- estradiol hormone according to Rajkowski *et al* (1977) and Joshi ,(1979) [ELISA technique]

2.4.8.1 Principle

17-β-Estradiol (antigen) in the sample competes with horseradish-peroxidase labeled 17 β-Estradiol (enzyme-labeled-antigen) for binding onto the limited number of anti 17 β-Estradiol (antibody) sites on the microplates (Solid phase).

After incubation, the free antigen is removed by washing. The enzyme substrate (H_2O_2) and the chromogen (TMB) are added. After incubation, the enzyme reaction is stopped and absorbance is read in a microplate reader.

17 β-Estradiol concentration in the sample is calculated based on a series of standards.

The colour intensity is inversely proportion to the 17 β-Estradiol Concentration in the sample.

2.4.8.2 Product contents

For 96 test assay

1) Microplate 12 x 8 micro well strips coated with anti-17-β-Estradiol Kg G.

   The plate is contained in a sealed bag with desiccant. Allow the plate to reach room temperature before use. To prevent any moisture formation inside the bag. Do not reuse wells.

2) Enzyme conjugate 1 vial of 0.4 mL. A 100 X concentrated protein buffer solution of 17 β-Estradiol-HRP conjugate.
3) Conjugate Diluent 1 vial of 30 ml. Contains phosphate buffer (50 mM. PH 7.4) and bovine serum albumin (1 g/L).
4) TMB-substrate 1vial of 12 ml. Contains TMB with activators and stabilizers diluted in a phosphate/citrate buffer.
5) Stop solution 1 vial of 12 ml. Contains warning avoid contact with eyes and skin.
6) Standards 4 vials of 1.0 ml. 17-β-Estradiiol ready to use standards of the following concentrations.

S1	S2	S3	S4	
10	60	400	2000	Pg/ml

7) Competitive solution 1 vial of 5 ml.

2.4.8.3 Procedure

As previously mentioned in page (70,71), however the differences include

The colour which develops in the last incubation is stable for a maximum of 30 minutes in the dark.

Assay Schematic

Anti-17 B-Estradiol Coated wells

Reagents	Blank	Bo	Standards	Sample
Conjugate diluent	-	100 µL	-	-
Standards	-	-	100 µL	-
Sample	-	-	-	50 µL
Competitive solution	-	-	-	50 µL
Diluted conjugate	-	100 µL	100 µL	100 µL

Cover strips with adhesive film.

Incubate for 120 minutes at +37°C.

Peel off adhesive film and aspirate the reaction solution from wells.

Wash 4-5 times with 300 µL/well of distilled or dionised water, carefully aspirating off the remaining liquid.

TMB substrate	-	100 µL	100 µL	100 µL

Cover strips with a new adhesive film.

Incubate for 30 minutes at room temperature (18-30°C), in the dark.

Stop solution	100 µL	100 µL	100 µL	100 µL

Read the absorbance at 450 nm against the blank (A1).

2.4.9 Estimation of progesterone according to Wisdom, (1976) and Hubl *et al.*, (1982) [ELISA technique]

2.4.9.1 Principle of Method

Anti-progesterone antibodies are immobilized on micro-well plates. Progesterone in the sample competes with HRP-labeled

progesterone to the immobilized antibody. After washing, enzyme substrate is added. The amount of progesterone in the sample is inversely proportional to the enzyme activity. The reaction is terminal by adding stopping solution. Absorbance is measured on a plate reader. The colour intensity is inversely proportional to the progesterone concentration in the sample.

2.4.9.2 Procedure As previousely mentioned in page (70,71),however there are some differences ,including

The colour developed in the last incubation is stable for at least 30 minutes in the dark.

Assay Schematic

Follow the Schematic below

Anti-progesterone IgG coated wells

Reagents	Blank	Standards	Sample
Standards	-	50 µL	-
Sample	-	-	-
Enzyme conjugate	-	50 µL	50 µL

Cover strips with adhesive film.

Incubate for 60 minutes ate 37°C.

Peel off adhesive film and aspirate the reaction solution from all wells.

Wash with 300ml/well of distilled water. Repeat wash by draining the water completely.

TMB solution	100 µL	100 µL	100 µL

Incubate for 15 minutes at room temperature, in the dark.

Stop solution	100 µL	100 µL	100 µL

Read the absorbance (E) at 450 nm against the blank (A1) within 30 minutes.

2.4.10 Estimation of parathyroid hormone (PTH) according to Orloffu and Steward.,(1989) [ELISA technique]

2.4.10.1 Assay Procedure

Allow all reagents to reach room temperature and mix thoroughly by gentle inversion before use. Assay standards, control and unknowns in duplicate.

Mark the microfiltration strips to be used.

Pipet 50 µl of standards, controls and unknowns to the appropriate wells.

Add 100 µl of the antibody blot in conjugate solution to each well using a semi-automatic dispenser.

Incubate the wells for 2-5 hours at room temperature (25°C).

Aspirate and wash each well five times with the wash solution using an automatic microplate washer. Blot it dry by inverting the plate on absorbent material.

All 100 µl streptovidin enzyme conjugate solution to each well using a semi-automatic dispenser.

Incubate the wells for 30 minutes at room temperature.

Aspirate and wash each well five times with wash solution using an automatic microplate washer. Blot it dry by inverting the plate on an absorbant material.

Add 100 µl of the TMB solution to each well (Tetramethyl benzidine) using a semi-automatic dispenser.

Incubate the wells for 20 minutes at room temperature (25°C). Avoid exposure to direct sunlight.

Read the absorbance.

Calculation

Calculate the mean absorbance for each standard, control or unknown.

Plot the log of the mean absorbance readings for each of the standards along the y-axis versus the log of the 1-PTH concentrations in pg/ml along the x-axis. Alternatively, the data can be plotted linear versus linear and a smooth spline curve-fit can be used.

Determine the 1-PTH concentration of the controls and unknowns from the standard curve by matching their mean absorbance readings with the corresponding 1-PTH concentrations.

2.4.11 Estimation of pH of urine

By using special urine strips and comparing the colour produced to the standard colour.

2.4.12 Statistical analysis

- Data were analysed using a computer (program, **Minitab (13)**).
- For comparison between means of 2 results, Students 't' test was used.

CHAPTER THREE

RESULTS

CHAPTER THREE

RESULTS

All results of the present study were tabulated in Tables (3-6) and were also illustrated in figures (4-17).

3.1 Data of all male groups

Comparison of parameters of group B_1 (early age group) with their corresponding data in group A_1 (children below 15 years age group) showed the followings in Table (3)

significant changes were found in serum calcium, phosphorus concentration ($P<0.05$) .

No significant changes were observed in TSH concentration and parathormone hormone , also PH of urine did not show any significant change

However, significant changes were observed in serum alkaline phosphatase concentration (negative $P<0.001$), solubility product (negative $P<0.01$), total T_3 (negative $P<0.05$), total T_4 (negative $P<0.05$), testosterone (positive $P<0.001$), progesterone (positive $P<0.05$).

Urine showed significant positive correlation in urine calcium and phosphorus concentration ($P<0.05$).

If the parameters of middle age group (C_1) are compared with their correspondings in early age group (B_1) it will be noticed that in Table (3)

Negative significant correlations were observed alkaline phosphatase enzyme ($P<0.01$), TSH ($P<0.01$), total T_3 ($P<0.01$) and total T_4

Table (3) All data of males in different age groups

Group	A1	B1		C1		D1	
Number	15	29		16		19	
Including	Children	Early age		Middle age		Old age	
Age range (yrs)	Below 15	20-35		35-55		55-72	
Blood parameters	**Mean ± SE**	**Mean ± SE**	**Pwith A_1**	**Mean ± SE**	**P with B_1**	**Mean ± SE**	**P with B_1**
1) Ca^{++} (mg/dl)	10.8 ± 0.25	9.9 ± 0.27	*	10.10 ± 0.33	*	10.7 ± 0.41	**
2) P^{---} (mg/dl)	5.6 ± 0.09	4.3 ± 0.3	*	4.73 ± 0.17	*	5.11 ± 0.12	**
3) Alk. Phosph. (IU/L)	252.8 ± 23.2	83.9 ± 5.01	***	72.70 ± 4.3	**	65.04 ± 3.7	***
4) Solubility product	60.48 ± 3.2	42.57 ± 2.9	**	47.77 ± 1.9	*	54.67 ± 2.8	**
5) TSH (μIU/ml)	4.2 ± 0.51	3.7 ± 0.4	NS	2.9 ± 0.34	**	7.8 ± 0.35	**
6) Total T_3 (ng/ml)	87.6 ± 4.3	92.6 ± 3.13	*	84.4 ± 3.7	**	80.7 ± 2.9	***
7) Total T_4 (ng/ml)	130.6 ± 11.8	138.95 ± 9.76	*	127.71 ± 8.10	***	119.41 ± 4.9	***
8) Testosterone (ng/ml)	1.178 ± 0.11	8.31 ± 0.34	***	6.07 ± 0.41	***	4.23 ± 0.27	***
9) Estradiol (ng/ml)	0.3 ± 0.02	0.47 ± 0.03	*	0.50 ± 0.01	*	0.51 ± 0.02	*
10) Progesterone (ng/ml)	0.11 ± 0.02	0.25 ± 0.02	*	0.33 ± 0.03	*	0.29 ± 0.014	*
11) Parathormone (ng/ml)	0.92 ± 0.07	1.2 ± 0.08	NS	1.8 ± 0.09	**	2.4 ± 0.09	***
Urine parameters 12) pH	4.9 ± 0.11	5.2 ± 0.17	NS	5.7 ± 0.18	NS	5.5 ± 0.20	NS
13) Ca^{++} (mg/dl)	2.8 ± 0.32	3.1 ± 0.19	*	6.7 ± 0.23	***	8.3 ± 0.31	***
14) P^{---} (mg/dl)	4.7 ± 0.27	5.3 ± 0.3	*	4.3 ± 0.31	**	3.8 ± 0.29	***

☛ where NS = Non Significant * = Significant (P<0.05)

= Highly significant (P<0.01)*= Very highly significant (P<0.001)

(P<0.001), estradiol (P<0.05), and testosterone (P<0.001). However, phosphate concentration in urine was found to be decreased significantly (P<0.01).

On the other hand, positive correlations were significantly observed in serum calcium (P<0.05) and in serum phosphorus (P<0.05), solubility product (P<0.05), estradiol (P<0.05), progesterone (P<0.05), parathyroid hormone (P<0.01). It was noted that urine calcium concentration was also significantly increased (P<0.001).

Comparison between data of the old age group (D_1) with their corresponding data of early age group (B_1), showed that all parameters were significantly changed as follows in Table (3)

Negative significant correlations were observed regarding serum alkaline phosphatase (P<0.001), TSH (P<0.01), total T_3 (P<0.001), total T_4 (P<0.001) and testosterone concentration (P<0.001). Also phosphate concentration in urine was significantly decreased (P<0.001).

Significant positive correlations were observed regarding serum calcium (P<0.01) and serum phosphorus (P<0.01) solubility product (P<0.01), progesterone concentration (P<0.05), estradiol level (P<0.05) and parathyroid hormone (P<0.001). In urine, concentration of calcium was significantly increased (P<0.001).

3.2 Data in all female groups

After comparing data of early age group (20-35 years old) (B_2) with below 15 years old group (A_2), it was noticed that in Table (4)

a) significant changes were observed in serum calcium (p<0.05) and serum phosphorus(P<0.05).

b) No significant changes were observed in TSH, total T_3, total T_4. Also, no significant changes were observed in concentration of calcium in urine and pH in urine.

c) There are significant negative correlation in serum calcium and phosphorus ($P<0.05$), alkaline phosphatase ($P<0.001$) and also in solubility products ($P<0.001$).

d) There are significant positive correlation in serum testosterone ($P<0.001$), estradiol ($P<0.001$), progesterone ($P<0.001$) and in parathormone ($P<0.05$).

By comparing data of middle age group (C_2) (35-55 years old) with their corresponding values in early age female groups (B_2), it was noticed that in the Table(4)

a) No significant effects were observed in urine pH, and serum calcium.

b) Significant negative correlations were observed in alkaline phosphatase ($P<0.01$), TSH ($P<0.01$), total T_3 concentration ($P<0.01$), total T_4 concentration ($P<0.001$), estradiol ($P<0.001$) and progesterone ($P<0.001$). Phosphorus concentration in urine was also significantly inecreased($P<0.001$). However, data showed significant positive correlation in serum phosphorus ($P<0.05$), solubility product ($P<0.05$), testosterone concentration ($P<0.001$) and in parathormone ($P<0.001$). Also calcium in urine was significantly increased ($P<0.001$).

Table (4) All data of females in different age groups

• Group	A_2	B_2		C_2		D_2	
• Number	16	98		49		18	
• Including	Children	Early age		Middle age		Old age	
• Age range (yrs)	Below 15	20-35		35-55		55-72	
• **Blood parameters** 1) Ca^{++} (mg/dl)	**Mean ± SE**	**Mean ± SE**	**P with A_2**	**Mean ± SE**	**P with B_2**	**Mean ± SE**	**P with B_2**
	10.4 ± 0.13	9.8 ± 0.21	*	9.9 ± 0.3	NS	10.3 ± 0.45	**
2) P^{---} (mg/dl(	5.4 ± 0.21	3.8 ± 0.24	*	4.7 ± 0.33	*	5.43 ± 0.43	**
3) Alk. Phosph. (IU/L)	261.7 ± 19.3	71.7 ± 5.5	***	60.4 ± 5.1	**	54.4 ± 3.9	***
4) Solubility product	56.16 ± 3.3	37.24 ± 2.8	***	46.53 ± 2.5	*	55.93 ± 3.6	***
5) TSH (µIU/ml)	4.3 ± 0.41	3.9 ± 0.29	NS	3.1 ± 0.20	**	2.6 ± 0.17	**
6) Total T_3 (ng/ml)	89.7 ± 5.31	93.2 ± 4.7	NS	83.3 ± 7.13	**	76.3 ± 6.7	***
7) Total T_4 (ng/ml)	125.3 ± 9.4	131.7 ± 8.1	NS	119.4 ± 7.9	***	103.4 ± 7.1	***
8) Testosterone (ng/ml)	0.2 ± 0.01	1.3 ± 0.01	***	2.1 ± 0.03	***	3.2 ± 0.02	***
9) Estradiol (ng/ml)	0.3 ± 0.01	6.2 ± 0.03	***	3.2 ± 0.01	***	0.77 ± 0.05	***
10) Progesterone (ng/ml)	1.3 ± 0.09	4.8 ± 0.27	***	2.2 ± 0.02	***	1.2 ± 0.01	***
11) Parathormone (ng/ml)	0.88 ± 0.05	1.3 ± 0.04	*	2.1 ± 0.07	***	3.2 ± 0.13	***
• **Urine parameters** 12) pH	5.1 ± 0.01	5.3 ± 0.02	NS	5.9 ± 0 03	NS	5.6 ± 0.04	NS
13) Ca^{++} (mg/dl)	3.4 ± 0.21	2.7 ± 0.21	NS	5.7 ± 0.23	***	7.1 ± 0.06	***
14) P^{---} (mg/dl)	4.5 ± 0.30	6.1 ± 0.17	***	5.3 ± 0.17	***	4.4 ± 0.04	**

☞ where NS = Non Significant * = Significant (P<0.05)

= Highly significant (P<0.01)*=Very highly significant

(P<0.001)Comparison between data of the old age group (D_2) (55-72 years old) are with date of early age group (B_2), it is noticed that in Table (4).

a) No significant change was noticed in pH of urine.

b) Marked significant negative correlations are noticed in serum alkaline phosphatase (P<0.001), total T_3 (P<0.001), total T_4 (P<0.001), estradiol (P<0.001) and progesterone (P<0.001). Urine shows significant decrease in urine phosphorus concentration (P< 0.01).

c) On the other hand, there are positive significant relations in serum calcium (P<0.01) and in serum phosphorus (P<0.01), solubility product (P<0.001), testosterone concentration (P<0.001) and in parathyroid hormone concentration (P<0.001). Urine also shows significant increase in calcium concentration (P<0.001).

Analysis of the above data of this study reveals the following

(1) Groups B_1 in male (early age) and B_2 in female are the only groups having normal concentration of sex hormones (testosterone in males and estrogen and progesterone in female) and other hormones (T_3, T_4, TSH and parathormone).

(2) Appearance of hormonal change starts from group C_1 in males and C_2 in females (middle age). However, these changes became more obvious and clear in groups D_1 in males and D_2 in females (old age groups).Calcium and phosphorus concentrations and also alkaline phosphatase.

showed normal concentration only in groups B_1 in males and B_2 in females: the groups which contain normal concentration of all hormones estimated.

(3) Hormonal changes are present in extreme age groups (group A_1, C_1 and D_1 in males and A_2, C_2 and D_2 in females). The only difference was between A_1 and groups C_1 and D_1 in males and the same in female was that, in old age, parathormone hormone is increased and both T_3 and T_4 are decreased in these groups.

(4) Although sex hormones are lowered in both early age groups and old age groups, osteoporosis occurs only in old age groups. This means that, sex hormones are not the only responsible hormones or factors responsible for osteoporosis present in old age groups of either sex.This also denotes that osteoporosis process is not due to deficiency of sex hormones but also due to effects of other hormonal factors and non-hormonal factors. It was noticed during taking personal history from each fellow, that, changes in parameters were more manifesting if the individual was obese and eating more fatty meals, or living a sedentary life. Table (5 .6) .

(5) Changes regarding calcium, phosphorus and alkaline phosphorus investigated in females are nearly the same changes present in males. The only difference is that, the changes in these minerals are more in females, which means that, osteoporotic effects present in old females are more than osteoporosis present in old males.

(6) Urine calcium concentration in group A_2 is more than that concentration in group A_1. On the other hand, its concentration in group B_2 is less than in group B_1, which means that the female sex hormones have a more hypocalcemic effect than male sex hormones. This might explain the fact that, bone loss in old women is more than in old men.

Table (5) Comparison between obese and non-obese old males of group D_1

• Group	D_1		
• Subgroup	Non-obese old males	Obese old males	
• Symbol	D_1a (n=8)	D_1b (n=10)	
• BMI (Kg/m^2)	**Mean ± SE**	**Mean ± SE**	**P with D_1a**
• **Blood parameters**	13.7 ± 1.19	31.27	***
1) Ca^{++} (mg/dl)	10.5 ± 1.19	11.30 ± 0.90	***
2) P^{-} (mg/dl(	5.03 ± 0.4	10.90 ± 0.87	*
3) Alk. Phosph. (IU/L)	71.77 ± 3.91	50.71 ± 3.91	***
4) Solubility product	52.8 ± 3.81	56.57 ± 3.84	**
5) TSH (μIU/ml)	8.5 ± 0.61	7.10 ± 0.41	**
6) Total T_3 (ng/ml)	87.5 ± 5.7	73.9 ± 3.91	**
7) Total T_4 (ng/ml)	131.31 ± 9.3	107.39 ± 7.5	***
8) Testosterone (ng/ml)	4.65 ± 0.28	3.81 ± 0.17	**
9) Estradiol (ng/ml)	0.43 ± 0.02	0.59 ± 0.02	**
10) Progesterone (ng/ml)	0.37 ± 0.01	0.21 ± 0.01	**
11) Parathormone (ng/ml)	1.9 ± 0.04	2.9 ± 0.05	***
• **Urine parameters**			
12) pH	5.9 ± 0.15	5.1 ± 0.30	*
13) Ca^{++} (mg/dl)	7.7 ± 0.23	8.9 ± 0.37	**
14) P^{---} (mg/dl)	3.5 ± 0.11	4.1 ± 0.27	***

☞ where NS = Non Significant * = Significant (P<0.05)

= Highly significant (P<0.01) *=Very highly significant (P<0.001)

Table (6) Comparison between obese and non-obese old females of group D2

• Group	D_2		
• Subgroup	Non-obese old males	Obese old males	
• Symbol	D_2a (n=8)	D_2b (n=10)	
• BMI	**Mean ± SE**	**Mean ± SE**	**P with D_2a**
• **Blood parameters**	12.7 ± 1.13	33.2 ± 1.90	***
1) Ca^{++} (mg/dl)	9.8 ± 0.56	10.8 ± 0.91	*
2) P^{-} (mg/dl(	3.15 ± 0.17	5.71 ± 0.23	**
3) Alk. Phosph. (IU/L)	58.4 ± 2.9	50.4 ± 2.41	**
4) Solubility product	30.87 ± 2.2	61.67 ± 3.91	***
5) TSH (μIU/ml)	2.9 ± 0.13	2.3 ± 0.11	**
6) Total T_3 (ng/ml)	80.7 ± 5.7	71.9 ± 3.8	***
7) Total T_4 (ng/ml)	10.8 ± 7.3	97.9 ± 5.4	***
8) Testosterone (ng/ml)	2.3 ± 0.15	4.1 ± 0.21	**
9) Estradiol (ng/ml)	0.79 ± 0.03	0.75 ± 0.03	*
10) Progesterone (ng/ml)	1.3 ± 0.09	1.1 ± 0.01	*
11) Parathormone (ng/ml)	2.7 ± 0.01	37 ± 0.21	**
• **Urine parameters** 12) pH	5.9 ± 0.28	5.3 ± 0.27	*
13) Ca^{++} (mg/dl)	7.0 ± 0.61	7.2 ± 0.39	NS
14) P^{-} (mg/dl)	4.0 ± 0.19	4.2 ± 0.13	NS

☞ where NS = Non Significant * Significant (P<0.05)

= Highly significant (P<0.01) *= Very highly significant (P<0.001)

Fig.(4): Serum calcium concentration in both males and females in all groups

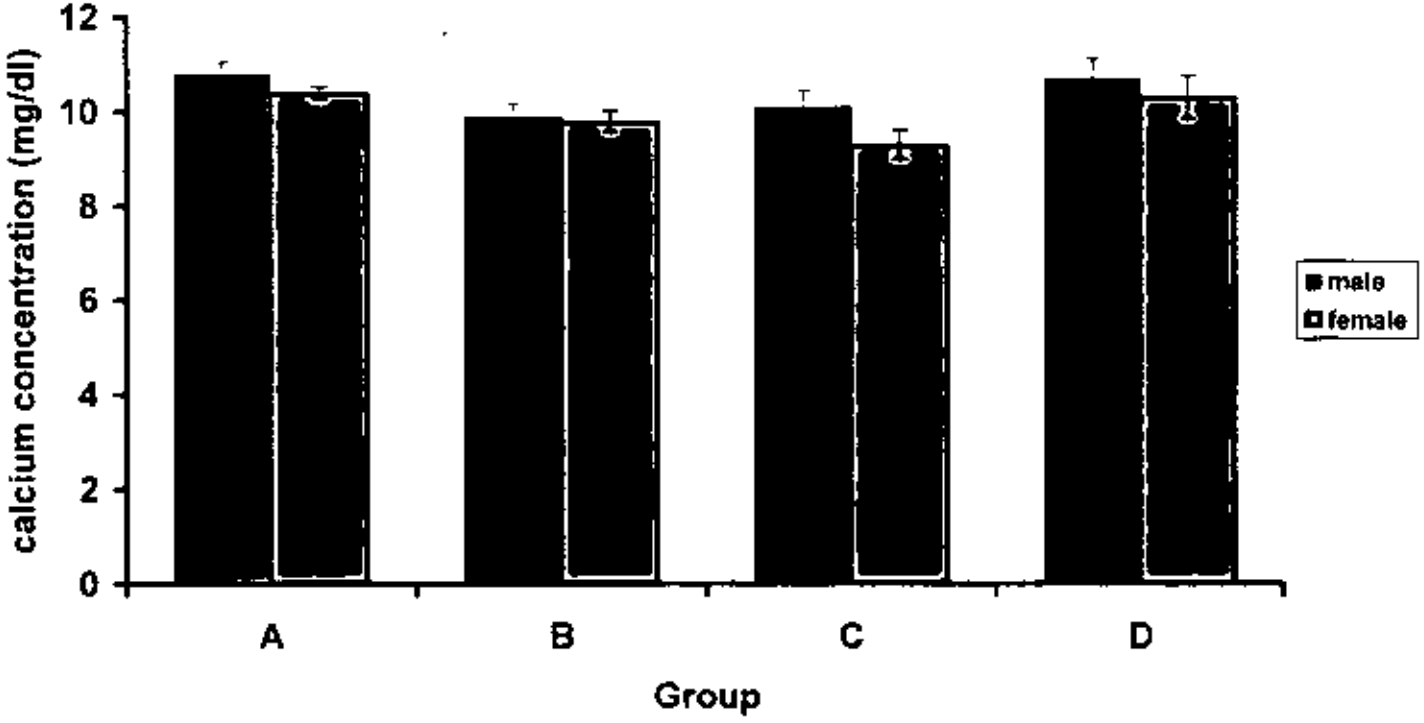


Fig.(5): Serum phosphorus concentration in both males and females in all groups

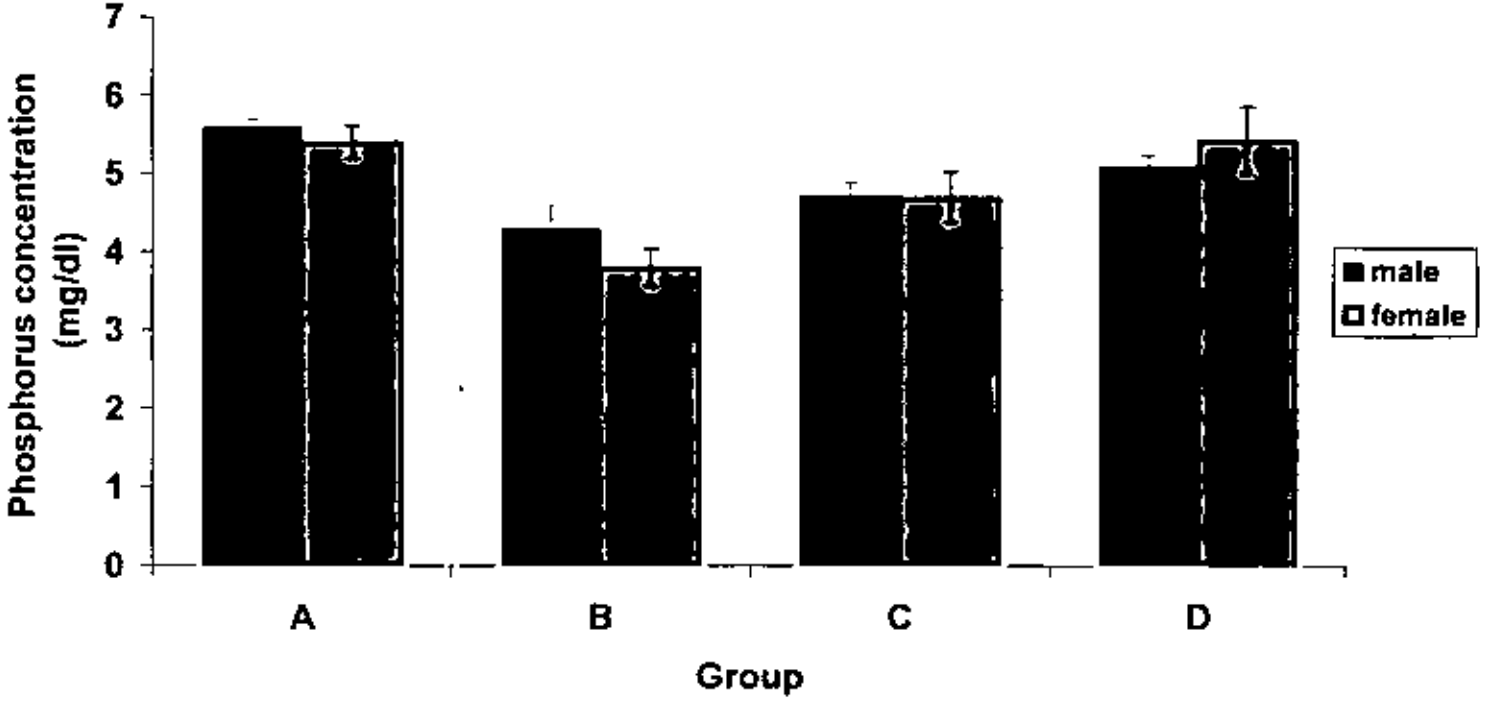


Fig.(6): Serum alkaline phosphatase concentration in both males and females in all groups

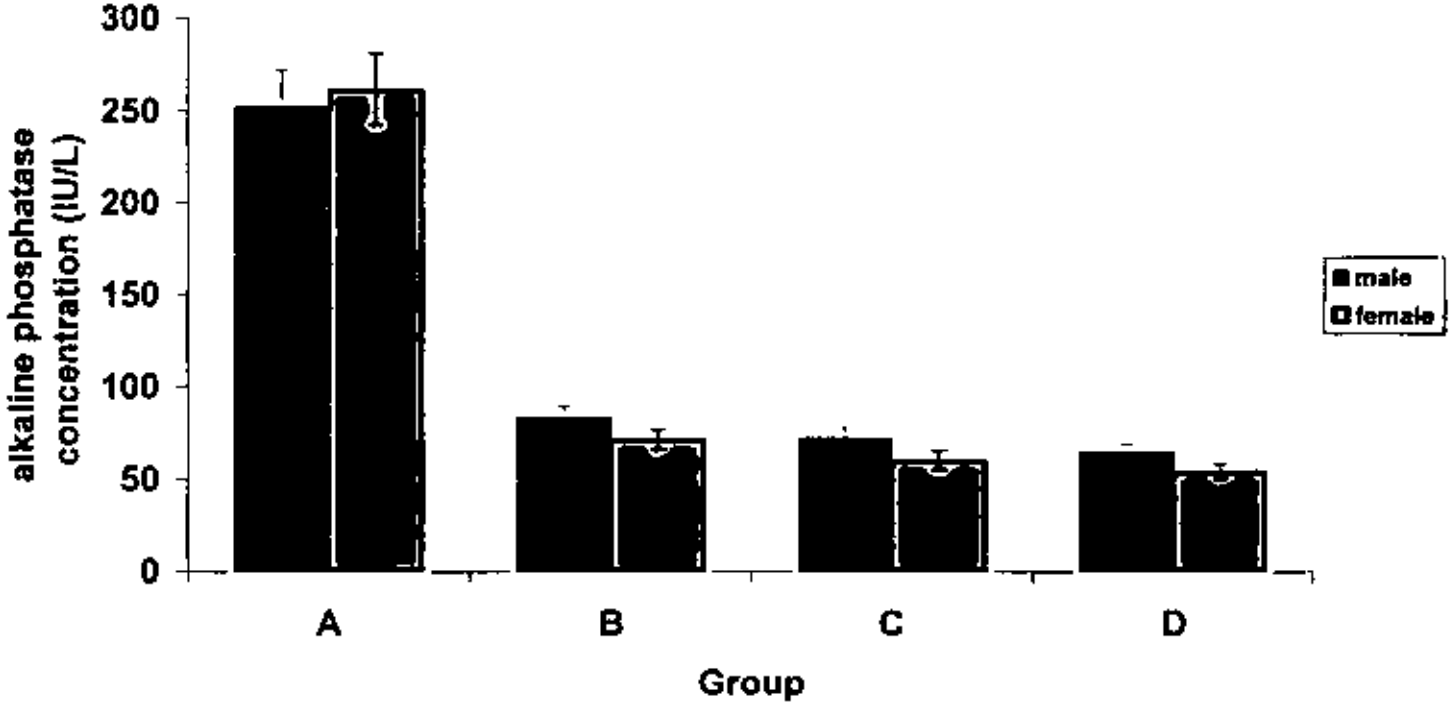


Fig.(7): Solubility products in all male and female groups

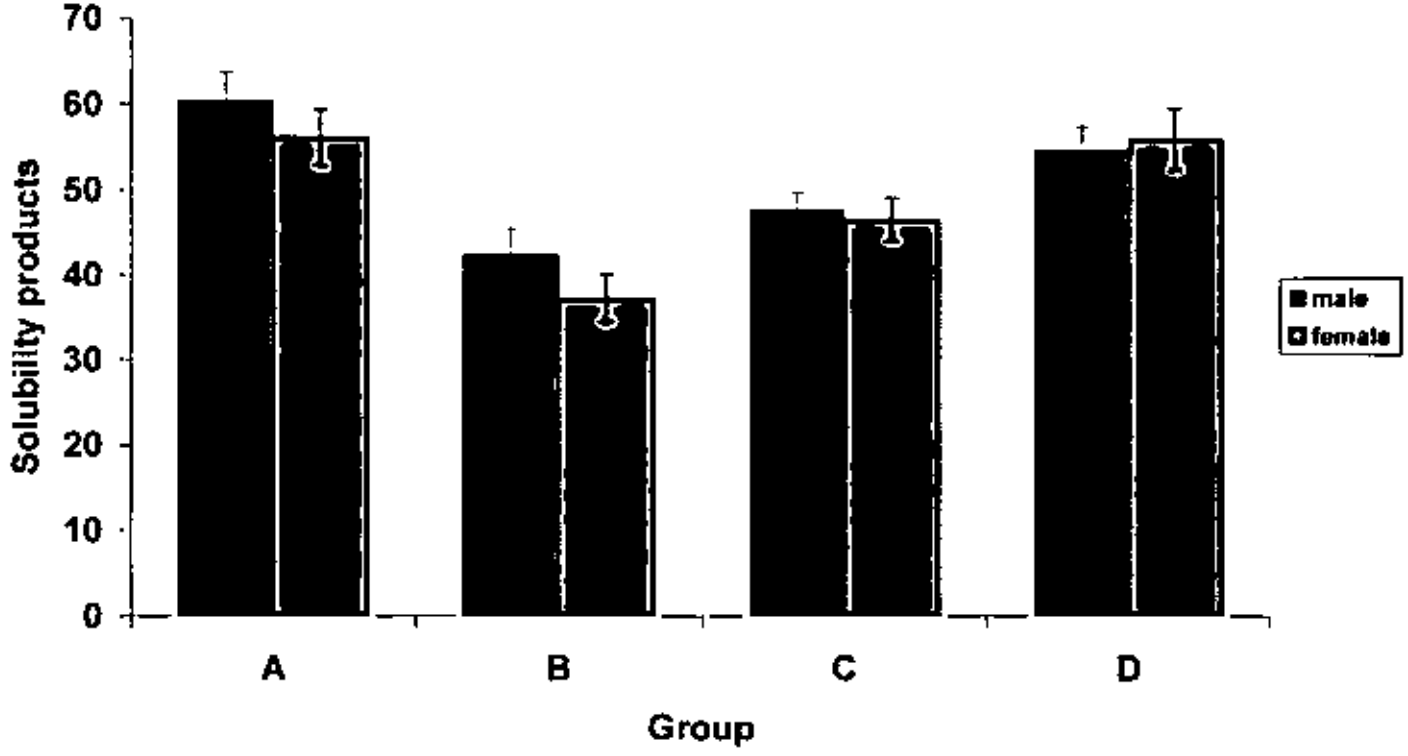


Fig.(8): Serum TSH concentration in all male and female groups

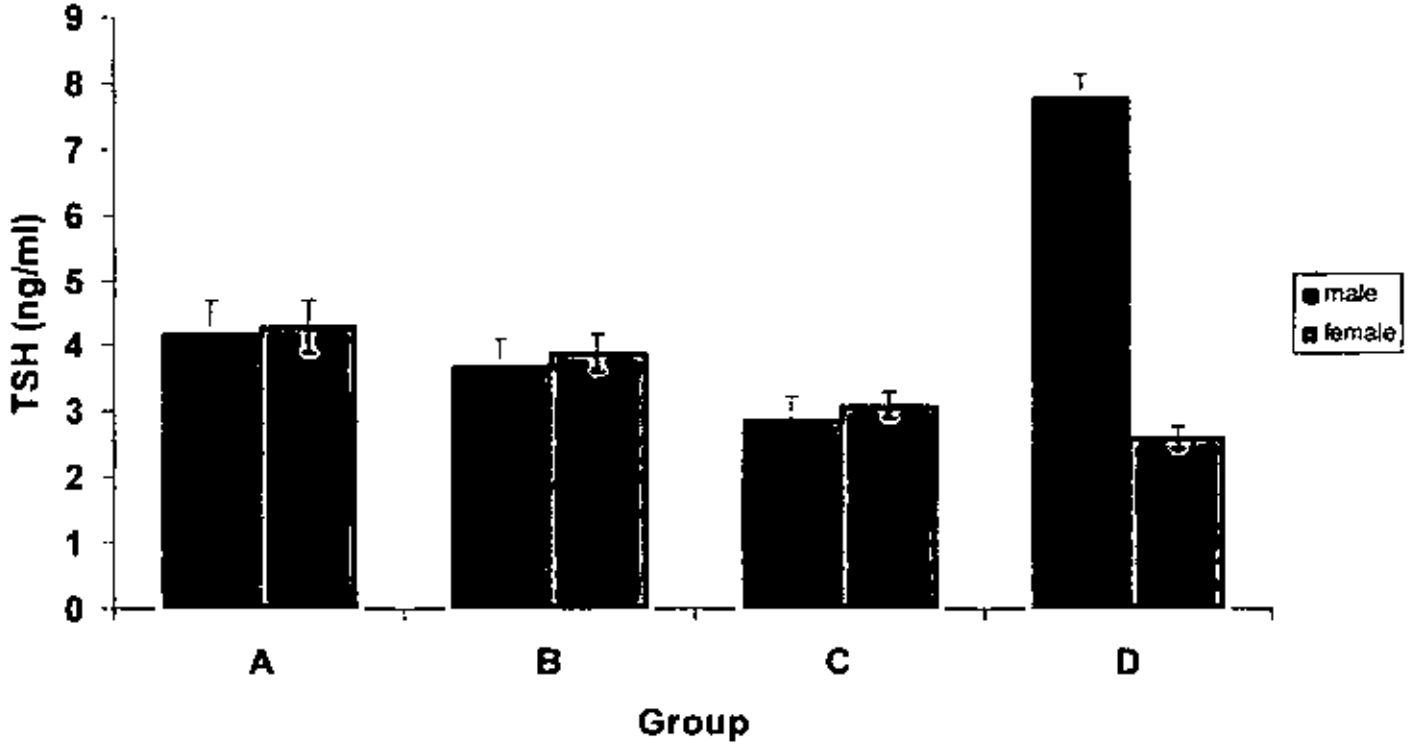


Fig.(9): Serum total T3 concentration in all male and female groups

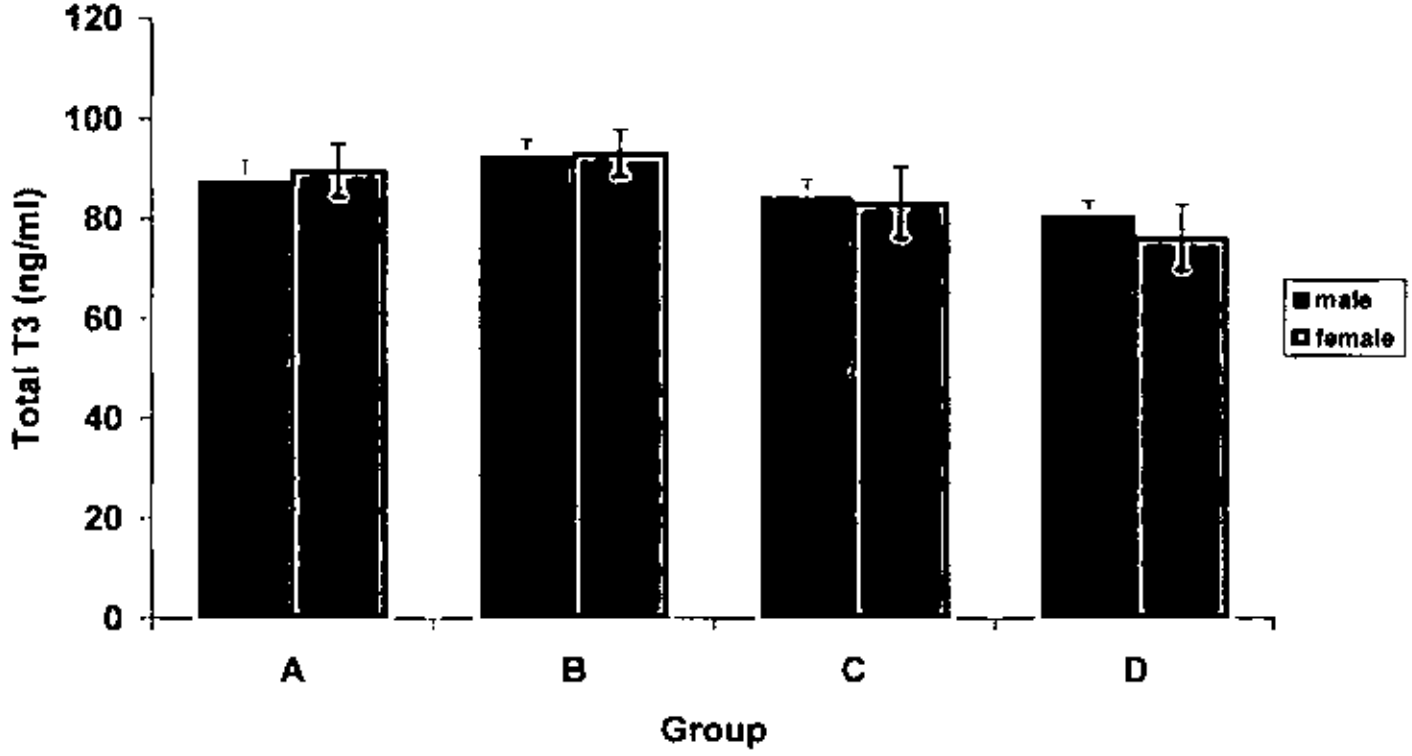


Fig. (10) :serum total T4concentration in all male and female groups.

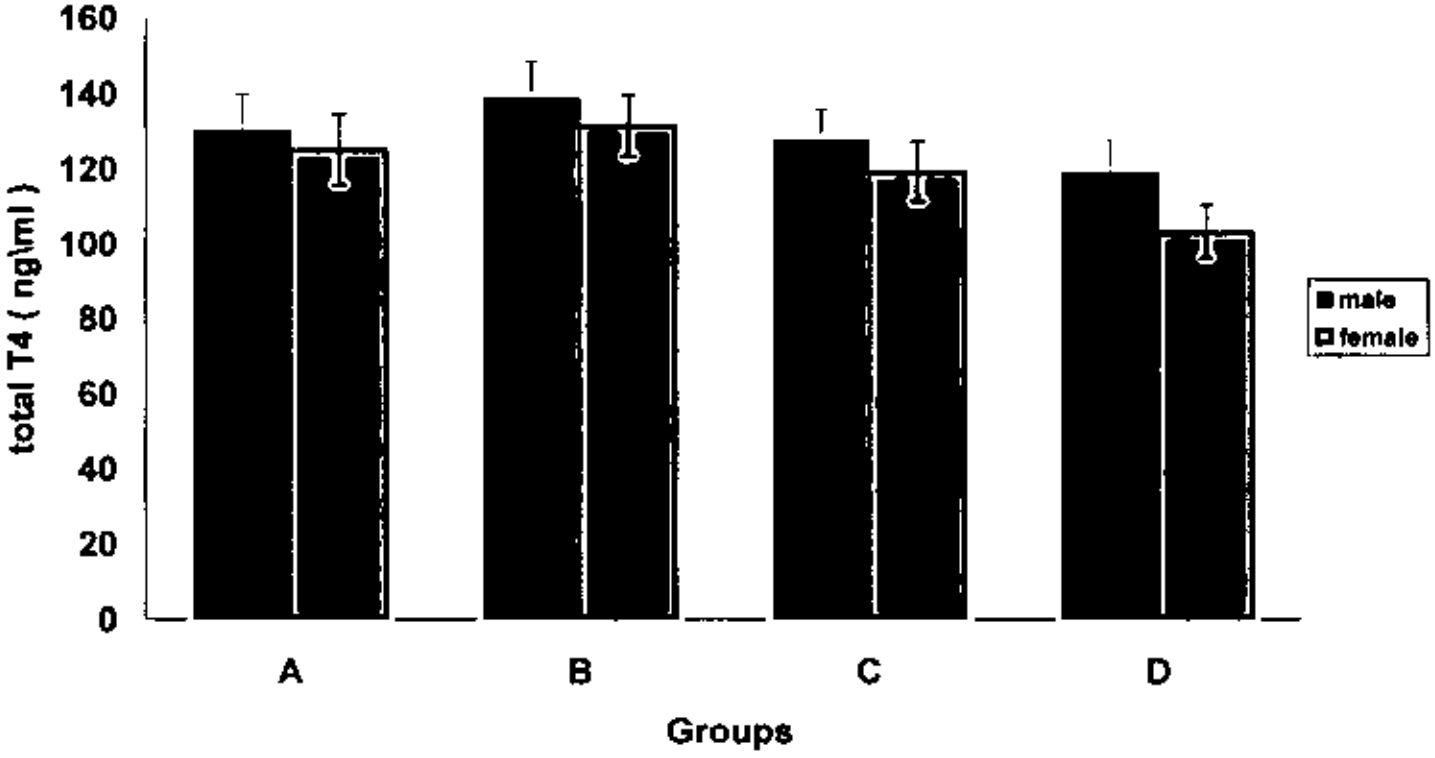


Fig.(11) :Serum testosterone concentration all male and female groups

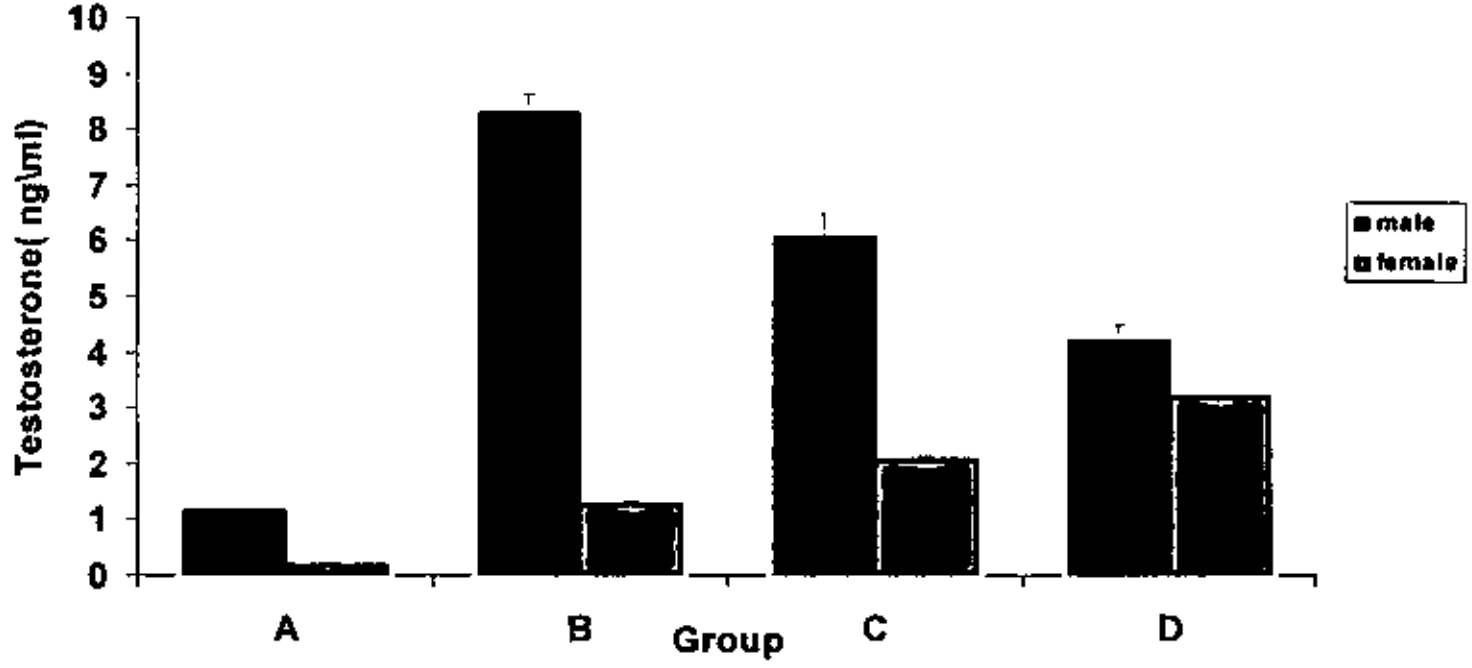


Fig. (12) :Serum estradiol concentration in all male and female groups

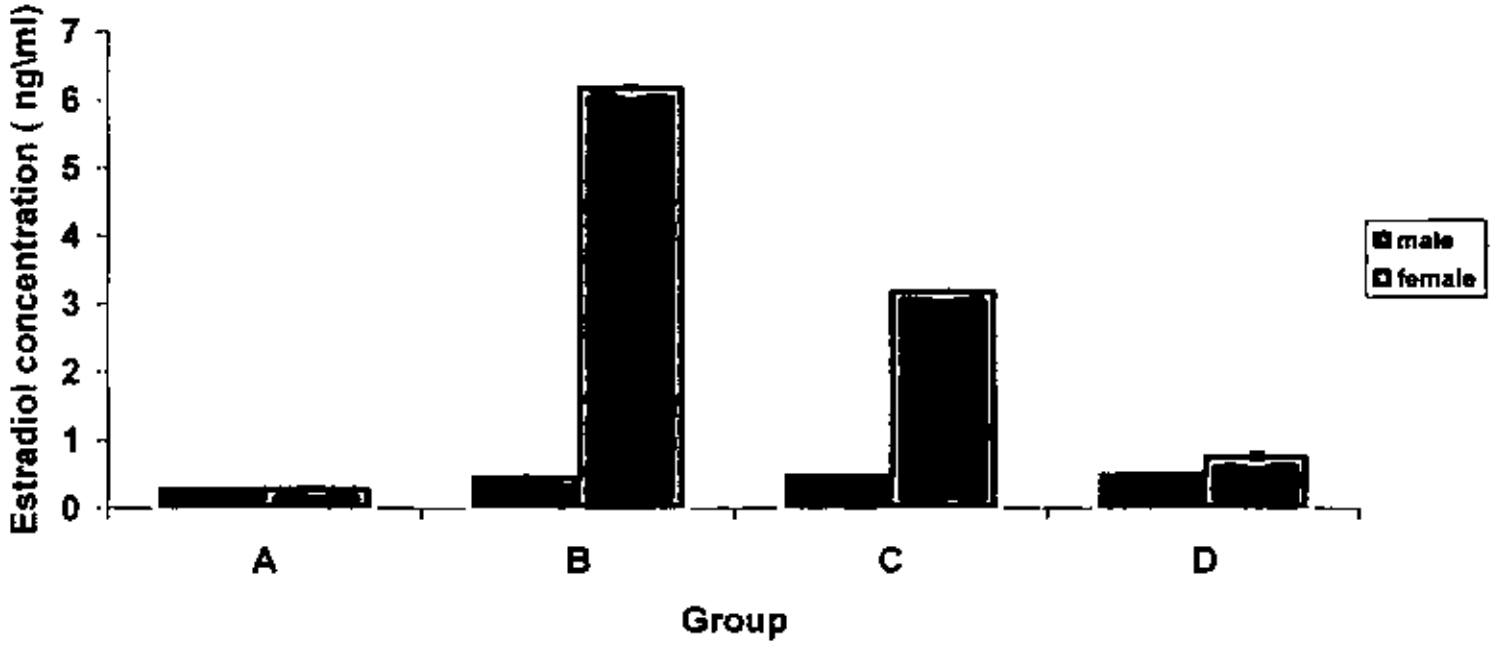


Fig. (13):Serum progesterone concentration in all male and female groups

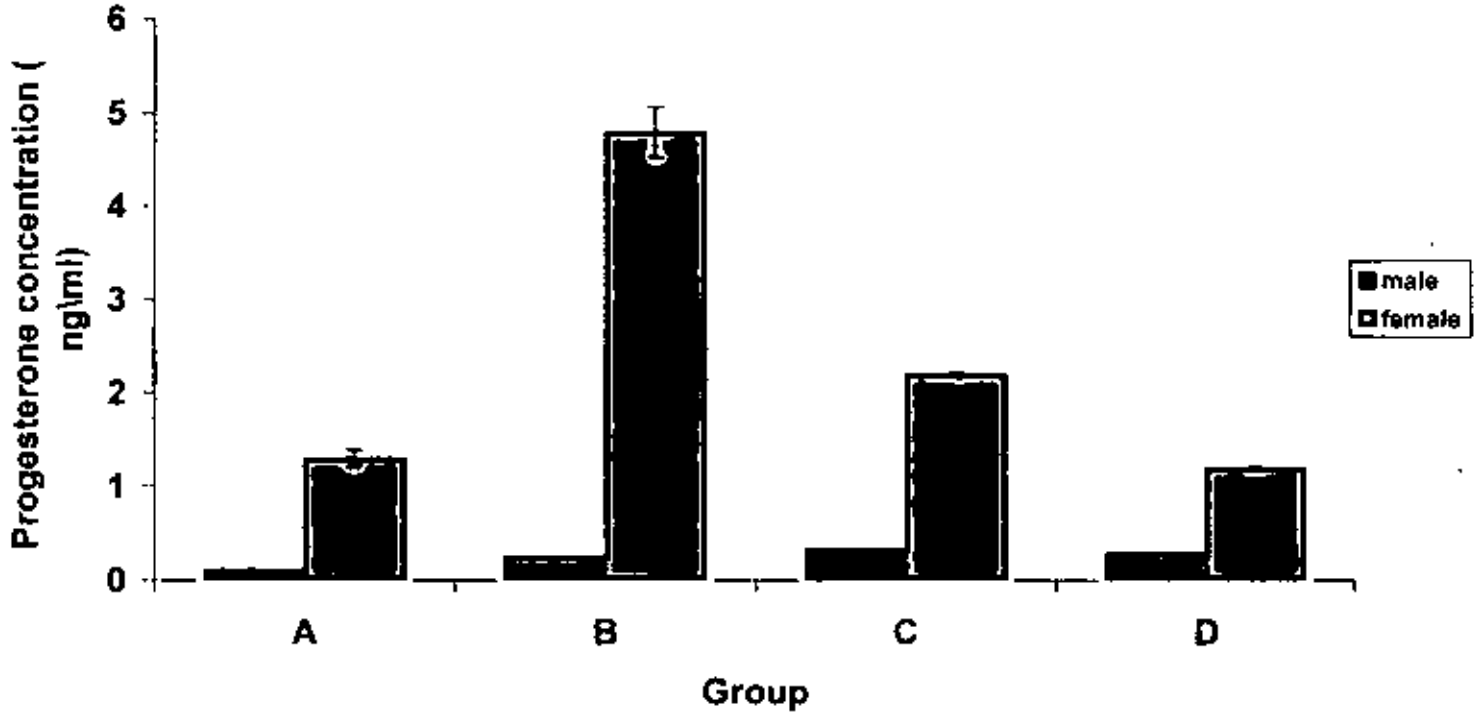


Fig. (14): serum parathyroid hormone concentration in all male and female groups

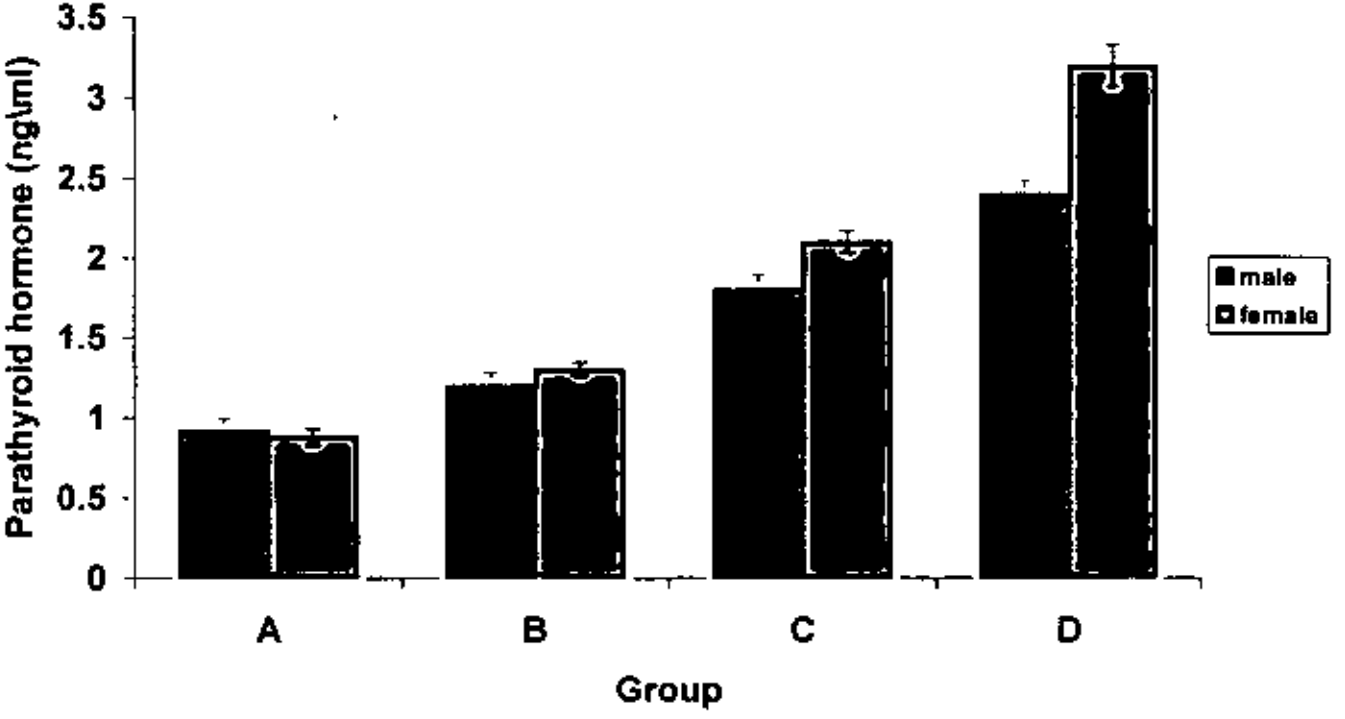


Fig. (15): pH of urine in all male and female groups

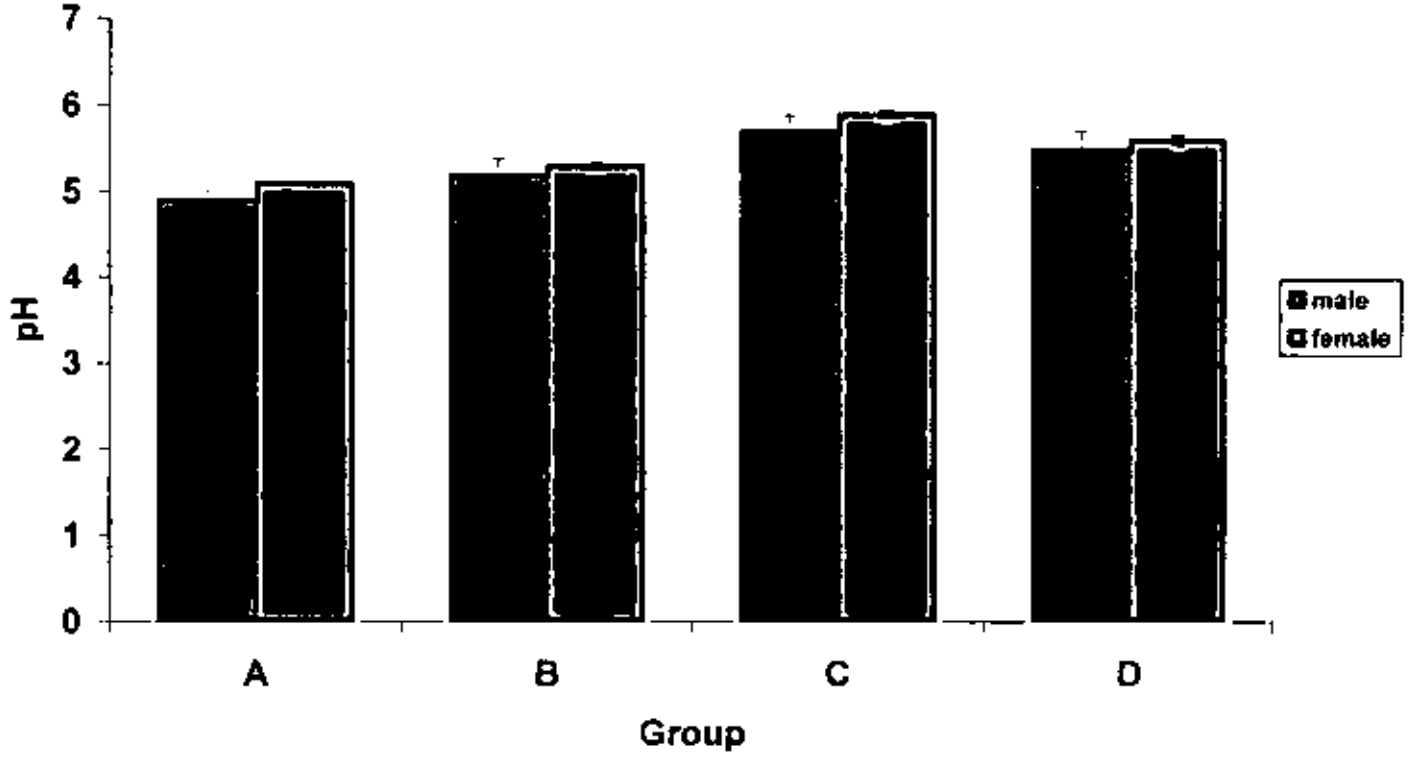


Fig. (16): Urine calcium concentraion in all male and female groups

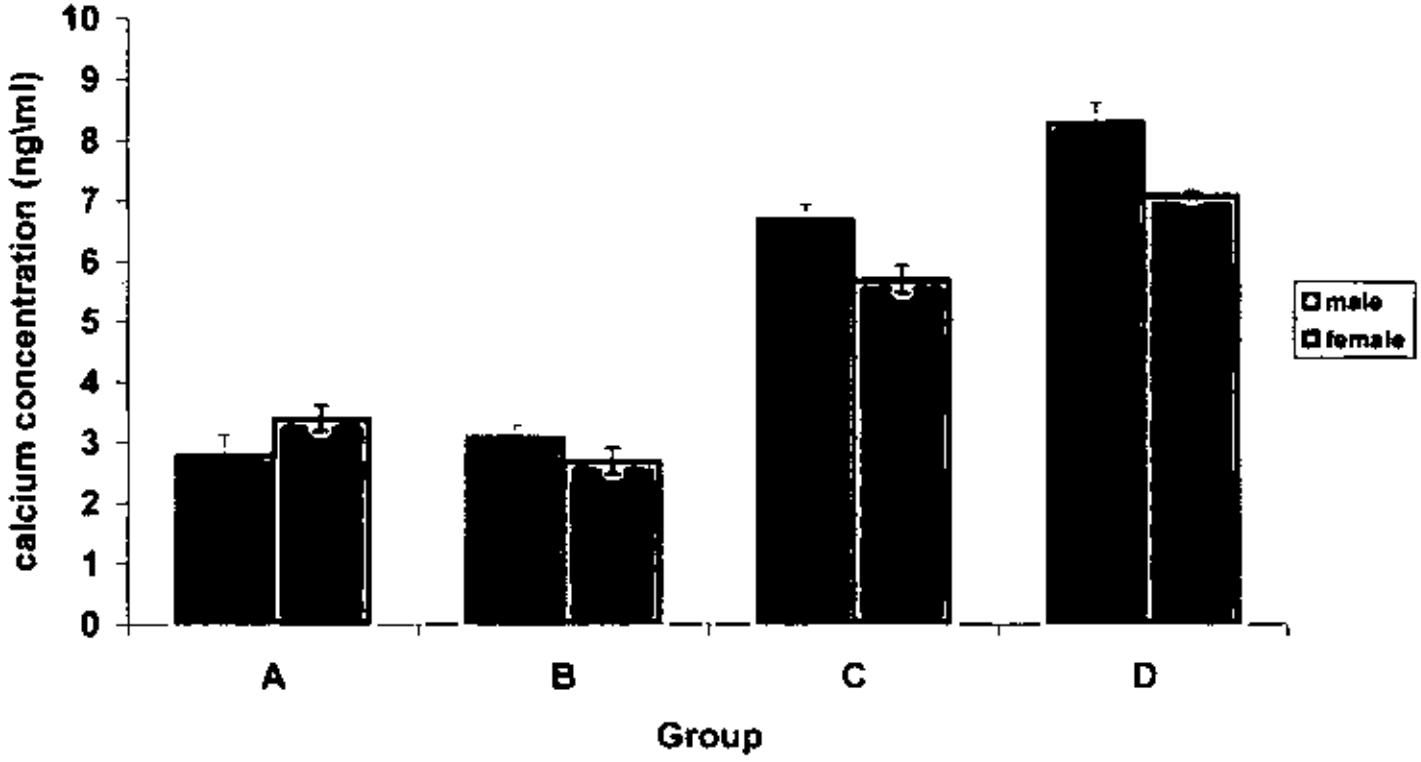
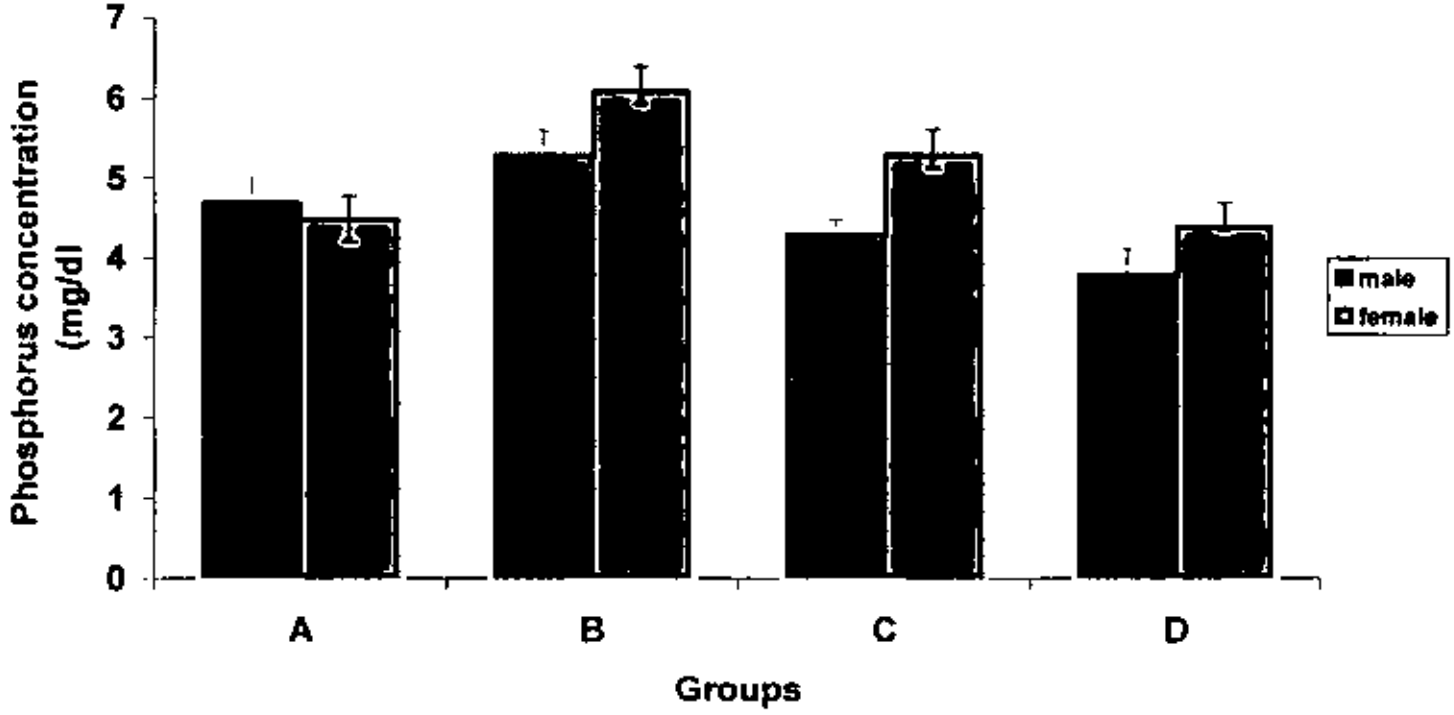


Fig. (17): Urine phosphorus concentration in all male and female groups



CHAPTER FOUR
DISCUSSION

CHAPTER FOUR

DISCUSSION

In this study, serum calcium, phosphorus, alkaline phosphatase and solubility product were significantly lowered in both males and females of members of group B; if they are compared with corresponding values in the fellows of group A. This means that sex hormones help in decreasing serum calcium and phosphorus concentration. In other words, sex hormones ; testosterone in males and estrogen and progesterone in females, have both hypocalcemic and hypophosphatemic effect. They also have an inhibitory osteoclastic activity and excitatory osteoblastic activity . Similar results were obtained by some investigators. **Schweikert *et al*** **(1980)** reported that androgens lower calcium and phosphorus levels, and this cannot be ascribed to renal waste, since calcium clearance and phosphate clearance do not increase.

Kasperk *et al.*, (1989) and **Vanderschueren *et al.*, (2004)** reported that androgens have a definite role in bone metabolism, calcium and phosphorus metabolism and enhance the hypocalcemic effect of thyrocalcitonin. This effect was noted as early as 12 hours after administration of testosterone. They based this finding on the currently prevailing view that the hypocalcemic effect of thyrocalcitonin is based on the direct inhibition of bone resorption. Thus, androgens may reflect an alteration in the serum calcium and phosphorus handling activity of bone. Moreover, they reported that the sensitivity to calcitonin was reduced after castration. In the doses used, long-term treatment with androgens increased the growth rate and thereby the response to

calcitonin in castrated rats, while the opposite was the case in intact male rats.

Cramer *et al.*, (1969) and **Douglas *et al.*, (1989)** noted that long term treatment with androgens enhances the hypocalcemic effect of calcitonin in castrated rats of either sex, but reduces the effect in intact animals.

The hypocalcemic effect and hypophosphatemic effect of androgens can be explained by a reduction in bone catabolism (osteoclastic activity) as there is no significant increase in enzyme alkaline phosphatase activity after androgen administration (**Fukayama and Tashjian ., 1989 and Kasperk *et al.*, 1989**).

However, **Cramer *et al.*, (1969)** and **Anderson *et al.*, (1997)** came to a conclusion that androgen treatment produces a fall in serum calcium and phosphorus levels without significant change in calcium clearance in tubular reabsorption of calcium. These observations are interpreted to mean that androgens decrease osteolysis without significant changes in any renal function. They also advised for use of androgens in patients suffering from disseminated metastasis in bone to decrease osteoporosis and also they should be used in men with idiopathic osteoporosis. Androgen might act by a mechanism that might involve estrogen.

Recently, **Colvard *et al.*, (1989)** and **Kasperk *et al.*, (1989, 1990)**, stated that, the mechanism by which testosterone can do its action on bone cells, involves that induction of transforming growth factor beta (TGF beta) and in addition, may sensitize the cells to show an enhanced response to fibroblast growth factor (FGF) and insulin-like growth factor II (IGF II).

To confirm this findings, alkaline phosphatase activity in the present study, was found to be lowered in old age group of either sex.

Contrary to our findings, are what reported by **Ayoub, (1963)**, who found that calcium and phosphorus in the serum of castrated lambs are lowered than non-castrated ones.

On the other hand, in the present study, oestrogen and progesterone were found collectively to decrease serum calcium and phosphorus. This effect seems to be a net result of multifactorial events including enhancing the osteoblastic activity and decreasing the osteoclastic activity of bones. They also decrease urinary calcium excretion. Therefore, oestrogen deprivation of after menopause causes rise in urine and serum calcium **(Eriksen *et al.*, 1988 and Dick *et al.*, 2004)**.

It was found that, oestrogen effects might be indirect. It might antagonizes parathormone action. Another hypocalcemic effect of oestrogen is the well known antagonizing estrogenic effect to growth hormone action, since the latter is hypercalcemic **(Gallagher *et al.*, 1973 and Lips *et al.*, 1989)**.

However, serum phosphate rises significantly after menopause.

Moreover, suggesting that oestrogen might decrease reabsorption from kidney **(Dick *et al.*, 2004 and John and Lee, 2006)** .

Regarding the osteoblastic stimulatory effect and the osteoclastic inhibitory effect of oestrogen on bone, recent studies stated that oestrogen inhibits secretion of cytokines such as interleukin I (IL-I), interleukin 6 (IL-6) and tissue growth factor α (TGF α). These cytokines foster the development of osteoclasts. Oestrogen also stimulates production of TGF B. These cytokines increase inhibition of osteoclasts. There are also

oestrogen receptors on osteoblasts and a direct stimulatory effect on them is a possibility **(Prestwook *et al.*, 1999; Ganong, 2001 and Dick, 2004)**.

This might explain the pathophysiology of osteoporosis happening in old age of both males and females.

It is well investigated that old women have less bone mass than old men, as women lose bone more rapidly than the men of comparable age **(Prestwook *et al.*, 1999; Francis, 2000 and Ganong, 2001)**.

This fact was achieved in the results obtained in this study .all previous results reported that to calcium and phosphorus were more manifested and significant in females more than males. **(Gallagher and Wilkinson ., 1973)**.

The calcium and oestrogen supplementation can reduce bone mass in postmenopausal women, suggesting an interaction between oestrogen deficiency and calcium balance **(Dick *et al.*, 2004 and Rosen, 2005)**.

It was also noted in this study that during taking personal history from all old person (males and females) that, those living a sedentary life, eating fatty meals and obese person showed marked more significant changes than data obtained from old thin, active or eating a balanced diet **(Anderson *et al.*, 1997 and Dick *et al.*, 2004)**.

This means that osteoporosis occurring in old age groups (males and females) is mostly a multifactorial process, and not only due to deprivation of sex hormones but might also be due to inappropriate diet intake, deficient digestion or absorption of calcium.

In this study, present group D individuals showed significant increase in parathyroid hormone(PTH). The mechanisms responsible for

this elevation of PTH with aging are yet unclear. Age related rise in PTH has been suggested to occur secondary to a decrease in functional renal mass (**Eriksen *et al.*, 1988**), a diminished intestinal calcium absorption (**Aurbach andChase ., 1978**), or an altered regulation of PTH release by serum calcium (**Douglas *et al.*, 1989 and Sosa *et al.*, 2000**).

It is surprising to know that, in old age, there is osteoporosis and hypercalcemia in presence of state of hyperparathyroidism. The excess PTH observed in old age beside other hormonal changes, might participate as a factor in pathogenesis of osteoporotic changes of olds.

Conclusively, the sex hormones (testosterone in males and oestrogen and progesterone in females) appears and seems to be major regulators of bone metabolism through their respective receptors mediated mechanisms (**Douglas, 1989 and Turner *et al.*, 1994**).

In the present study, it was found that total T_3 and T_4 concentrations were significantly decreased in postmenopausal individuals in both sex. The deficient T_3 and T_4 increases the production of the bone growth factor, insulin-like growth factor (IGF I), in osteoblastic cells and that it promotes the production of the osteoclastogenic cytokines, interleukin-6 (IL-6) (**Williams *et al.*, 1973 and Dick *et al.*, 2004**). This might be an additional factor that might participate in the pathogenesis of osteoporosis occurring in olds of either sex.

From the above discussion, it could be concluded that

(1) Sex hormones; testosterone in males and oestrogen and progesterone in females, have a definite decreasing effect on serum calcium and phosphorus.

(2) These sex hormones have a definite effect on the renal handling of calcium and phosphorus as calcium and phosphorus concentrations in urine have been changed in extreme age groups (A + D) which have inadequate sex hormones.

(3) Moreover, sex hormones have a significant effect on metabolism of bone and calcium and phosphorus. Their effects on bone are in part stimulatory to osteoblastic activity (anabolic effect) and inhibitory to osteoclastic activity (anticatabolic). This can be seen in the different alterations in activity of alkaline phosphatase enzyme. Its activity is increased in children but decreased in old age groups.

(4) The osteoporotic changes occurring in old age seems to be a multifactorial event. It might be due to
   a) Deficient sex hormones of both sexes.
   b) Deficient T_3 and T_4.
   c) Excess PTH.
   d) Inability or lack of exercise.
   e) Increase in body weight.
   f) Bad dietary habits and improper digestion.

(5) Data obtained from females run parallel to data obtained from males. There are only two differences
   a) Differences related to hormonal changes.
   b) Data of female groups are more significant than data of male groups. This might give an explanation to bone loss in old women is more than in men.

(6) The previous fact, might denote that the effects of female sex hormones (oestrogen and progesterone) on bone and calcium

metabolism is much more effective than the effects of androgens on these elements.

REFERENCES

REFERENCES

Albright F and Reifenstein EC Jr. (1993) The parathyroid glands and metabolic bone disease. Baltimore, Williams and Wilkins. pp. 40-45.

Amins S. Yuquing Z. Clark T (cited in Williams Textbook of Endocrinology,2002). (2000). Association of hypogonadism and oestradiol level with bone mineral density in elderly men from the Framingham Study. Annual Internal Medical. 122 951-63. (Abstract).

Anderson FH. Francis RM. Peaston RT. Wastell JH. (1997) . Androgen supplementation in eugonadal men with osteoporosis : Effect of six month's treatment on markers of bone formation and resorption. Journal of Bone and Mineral Research. Mar; 12(3) 472-8. (Abstract).

Anderson FH. Francis RM. Selby PL (cited in Williams Textbook of Endocrinology, 2002), (1998) Sex hormones and osteoporosis in men. Calcify Tissue Internal. 62 : 185-8 (Medline).

Armbrecht H (1986) Age-related changes in calcium and phosphorus uptake by rat small intestine. Biochemical Biophysical Acta. Jul 16: 882(3) : 281-6.

Atman PL. Dittmer DS (eds). (1968) Excretion products in sweat man. Metabolism-Biological Handbook. P. 519.

Aurbach GD and Chase LR. (1978) Cyclic 3'5 Adenylic Acid in bone and the metabolism of action of parathyroid hormone Fed. Proceedings on the National Academy of Sciences.

Ayoub MH (cited in Williams Textbook of Endocrinology, 2002). (1963) Calcium inorganic phosphorus and magnesium in serum of Egyptian lambs. Biochemistry Secretory Veterinary Research Laboratory. Dokki. Cairo. 525-29.

Bank N (cited in Williams Textbook of Endocrinology, 2002). (1974) A microperfusion study of phosphate reabsorption by the rate of proximal renal tubule. Effect of parathyroid hormone. The Journal of Clinical Investigation. 54 : 1040.

Barneett A. Wasserman RH (1973) Control of calcium absorption an dintestinal calcium-binding protein synthesis. Biochemical Biophysical Research Common. 54 : 191-196.

Barengolts El, Kouznetsova T, Segalene A, Lathon P, Odvina C, Kukreja Unterman TG (1996) . Effect of progesterone on serum levels of IgG-1 and femur IgF-1 mRNA in ovariectomized rats. The Journal of Bone Mineral Research, Oct; 11(10) : 1406-12. University of Illinois Medical Centers, Chicago, USA.

Bernard G (cited in Williams Textbook of Endocrinology, 2002), (1963) Calcium and phosphate metabolism in patients with disseminated breast cancer . Effects of Androgens and of Prednisone. Vol. 23, 1115-24.

Bijvoet Alvioli LV, Krane SM (ed), (1977) Kidney function in calcium and phosphate metabolism, in alveoli, L.V. Krane, S.M. (ed). Metabolic Bone Disease, Vol. I, New York. Academic Press. Pp. 49-140.

Blum JW, Mayer GP and Pott JT Jr, (1974) Calcium transport through cell membrane. Endocrinology, 95: 81.

Boland AR, Nemere I, Normal AW, (1990) Ca^{2+} channel agonist BAY K 8644 mimics 1.25 $(OH)_2$-vitamin D_3 rapid enhancement of Ca^{2+} transport in chick perfused duodenum. Biochemical Biophysical Research Commun. 166: 217-222.

Borle AB, (1967) Membrane transfer of calcium. Clinical Orthopedic Related Research. 52: 267-291.

Borle AB, Keutmann HT, Neumann WF, (1963) Role of parathyroid hormone in phosphate transport across rat duodenum.The Journal of American Medical Physiology, 204 : 705.

Boudry JR (cited in Williams Textbook of Endocrinology, 2002), (1975) Secretion of inorganic phosphate in the rat nephron. Clinical Society. 48 475.

Brook C, and Marshall N, (2001) Essential Endocrinology. Fourth Edition. Blackwell Science Ltd., London.

Brown EM, Pollak M, Hebert SC, (1998) The extracellular calcium sensing receptor. It's role in health and disease. Annual Reviews Medical. 49 : 15-29.

Burke D (cited in Williams Textbook of Endocrinology, 2002), (1978) Laboratory studies when to act on unexpected test result patient. Care. 12:14-87.

Carmer CF. (1965) Effect of Ca^{2+}/P^{---} ratio and pH on calcium and phosphorus absorption from dog gut loops in vivo. Canadian Journal of Physiology and Pharmacology. 43 : 75.

Cavalieri RR. Rappaport B. (1977) Impaired peripheral conversion of thyroxine to triiodothyronine. Annual Reviews Medical. 28 : 57-65.

Caverzasio J. Rizzoli R, Bonjour JV. (1986) Sodium dependent phosphate transport inhibited by parathyroid hormone and cyclic AMP stimulation in an opossum kidney cell line. Biological Chemistry. 261: 3233-3237.

Chopra J. Solomon DH and Ho RS. (1977) A radioimmunoassay of triiodothyronine. Clinical Endocrinology. 33 : 865.

Clark I. Rivera-Cordero F. (1971) Effect of parathyroid function on absorption and excretion of calcium magnesium and phosphate by rats. Endocrinology. 88 : 302.

Clark NB. Dantzler WH. (1972) Renal tubular transport of calcium and phosphate in snakes. A role of parathyroid hormone. American Journal of Medical Physiology. 223 : 1455.

Colvard DS. Erikson EF. Keeting PE (cited in Williams Textbook of Endocrinology. 2002). (1989) Identification of androgen receptors in normal human osteoblast-like cells. Proceedings of the National Academy of Science. USA. 86 : 854-857. [Abstract]

Conigrave AD. Quinn SJ. Brown EM. (2000) L-amino acid sensing by the extracellular Ca^{2+} sensing receptor. Proceedings of the National Academy of Science. USA. 97 : 4814-4819.

Corradino RA. (1973) Embryonic drick intestine in organ culture. A unique system for the study of intestinal calcium absorptive mechanism. The Journal of Cell Biology. 58 : 64.

Corvillain J. Abramow M. (1962) Some effects of human growth hormone on renal hemodynamics and on tubular phosphate transport in man. The Journal of Clinical Investigation. 41 : 1230.

Corvillain J. Abramow M. (1972) Growth and renal control of plasma phosphate. The Journal of Clinical Endocrinology and Metabolism. 34 :452.

Cramer CF. (1965) Effect of calcium and phosphate ratio from dog gut loops in vivo. Can. The Journal of Physiology and Pharmacology. 43 : 75.

Cramer CF. Copp DH. (1959) Progress and rate of absorption of radiostrontium through the intestinal tracts of rats. Proceedings of the Society of Experimenal Biological Medicine. 102 : 514-17.

Cramer CF, Parks CO, Copp DH, (1969) The effect of chicken and dog Calcitonin on some parameters of calcium, phosphorus and magnesium metabolism in dogs. Can. The Journal of Physiology and Pharmacology. 47 : 181.

Daily WH, Tonnesen AS, Allen SJ, (1990) Hypophosphatemia incidence, etiology and prevention in the trauma patient. Critical Care Medicine. 18 1210-1214.

Daly A, Ertingshausen G, (1972) Clinical Chemistry. 18 : 263-565.

Dawson-Hughes B, Harris SS, Krall EA (cited in http \www.pubmed .gov) (1997) Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age and older. The New England Journal of Medicine. 337 : 670-6.

Dick A, Devine J, Beilby and Prine RL, (2004) Effect of endogenous estrogen on renal calcium and phosphate handling in elderly women. The Journal of American Physiology. Endocrinology and metabolism.140. 115-157.

Douglas-Colvard, Frik F. Eriksen, Philip E. Keeting, Elizabeth M. Wilson, Denis B. Lubhan, Franks S, French B. Lawrence Riggs and Thomas C. Spelsberg, (1989) Identification of androgen receptors in normal human osteoblast-like cells. National Academy of Sciences. Vol. 86, No. 3, 854-857 by National Academy of sciences .

Dufour DR, Lott JA, Nolte FS (cited in Williams Textbook of Endocrinology, 2002). (2000) Diagnosis and monitoring of hepatic injury. Part I Performance Characteristics of Laboratory Tests. Clinical Chemistry, Vol. 46, NO. 12, p. 2027-2049.

Eisenberg E (1965) Effects of serum calcium level and parathyroid extracts on phosphate and calcium excretion in hypoparathyroid patients. The Journal of Clinical Investigation. 44 : 942-946.

Eisenberg E. (1968) Renal effects of parathyroid hormone in Talage, RV, Belanger LF parathyroid hormone and thyrocalcitonin.(Calcitonin). Amsterdam. Excerpta Medica. p. 465-475.

Eisenberg E In Pincus G and E.P. Vollmer. (1964) Biological activities of steroids in relation to cancer. Academic Press, New York and London. P. 189.

Elin RJ, Armstrong WD, Singer L, (1971)Body fluid electrolyte composition of chronically magnesium deficient control rats. American Journal Medical Physiological. 220 : 543-548.

Elin RJ, Armstrong WD, Singer L, (1994) Body fluid electrolyte composition of chronically magnesium-deficient and control rats. American Journal of Medical Physiological. 373 : 603-609.

Engler H, Oettli RE, Riesen WF, (1999) Biochemical markers of bone turnover in patient with thyroid dusfunctions and in euthyroid controls. A cross-sectional study. Clinical Chimeal Acta. 298 : 159-172.

Epstein, Kiechic FL, Artiss JD (cited in Williams Textbook of Endocrinology, 2002), (1986) The clinical use of alkaline phosphatase enzymes. Clinical Laboratory Medical. Vol. 6, No. 3, p. 491-505.

Eriksen EF, Colvard DS, Berg NJ, Graham ML, Mann KG, Spelsberg TC and Riggs BL, (1988) Estrogen resceptors in bones. Science. 241 84-86.

Falahai-Nini A, Riggs BL, Atkinson EJ (cited in Williams Textbook of Endocrinology, 2002), (2000) Relative contribution of testosterone and estrogen in regulating bone resorption and formation in normal elderly men. The Journal of Clinical Investigation. 106 : 1553-1560.

Fischer JA, Blum JW and Binswanger UJ, (1973) The Journal of Clinical Investigation. 52 : 2434.

Francis RM, (2000) Male osteoporosis. Rheumatology. 39 : 1055-1057. (Full Text).

Friedman PA, Gesek FA, (1995) Cellular calcium transport in renal epithelia measurement, mechanisms and regulation. Physiology Reviews. 75 : 429-471.

Fukayama H, Tashjian AHD, (1989) Direct modulation by androgens the response of human bone cells (SAOS-2) to human parathyroid hormone (PTH) and PTH related protein. Endocrinology. 125 : 1789-1794.

Fullmer CS, (1992) Intestinal calcium absorption calcium entry. American Journal of Clinical Nutrition. 122 : 644-650.

Gallagher FC. Nordin BEC in Van Keep, P.A. Lauritzen C (ed) , (1973) Ageing and estrogens. Frontiers of Hormone Research Basal Karger. P. 98.

Gallagher JC, Wilkinson R. (1973) The effect of ethinyloestradion on calcium and phosphorus metabolism of post-menopausal women with primary hyperparathyroidism. Clinical Sciences of Molecular Medicine. 45 : 782-785.

Gamst O. Try K. (1980) Scand. The Journal of Clinical Laboratory Investigation. 40 : 483-486.

Ganong MD. (2001) Review of Medical Physiology 20th Edition. Lange Medical Book.

Gill JR Jr, Casper AGT. (1971) Renal effects of adenosine 3'5'-cyclic monophosphate. Evidence for a role adenosis 3'5'-cyclic monophosphate in the regulation of proximal tubular sodium reabsorption. The Journal of Clinical Investigation. 50 : 1231.

Goodschalk M, Levy JR, Downs Jr., (1992) Glucocorticoids decrease vitamin D receptor number and gene expression in human osteosarcoma cells. The Journal of Bone and Mineral Research. 7 : 21-27

Goussous R. Song L. Dallal GE. Dawson Hughes B. (2005) Lack of effect of calcium intake on the 25-hydroxyvitamin D response to oral vitamin D_3. The Journal of Clinical Endocrinology and Metabolism. 90: 707-711.

Grant FD. Conlin PR. Brown EM. (1990) Rate and concentration dependence of parathyroid hormone dynamics during stepwise changes in serum ionized calcium in normal humans. The Journal of Clinical Endocrinology and Metabolism. 71 : 370-378.

Greenspan SL, Greenspan FS. (1999) The effect of thyroid hormone on skeletal integrity. Annual International Medical. 130 : 750-758.

Guyton and Hall. (2001) Textbook of Medical Physiology, 10th Edition. Saunders Company.

Harrison HE. Harrison HC. (1961) Transfer of $Ca^{2+}45$ across intestinal wall in wall in vitro in relation to action of vitamin D and cortisol. American Journal of Medical Physiology. 199 : 265-271.

Heaney RP. (1986) Bone and Mineral Research. Vol.4. Elsevier. New York. Pp. 255-301.

Hebener JF and Potts FT Jr., (1976) Endocrinology. 98 : 197.

Heersche JN, Bellows CG, Ishida Y. (1998) Progesterone and osteoporosis. The decrease in bone mass associated with aging and menopause. The Journal of Prosthetic Dentistry. Jan. 79(1) 14-6.

Helenius T, Tikanoja S.(1986) Sensitive and practical immuno-radiometric assay of thyrotropin. Clinical Chemistry. 32 : 514-518.

Hill PA, Reynolds JJ, Meikle MC, (1995) Osteoblasts mediate insulin-like growth factor I and II stimulation of osteoclast formation and function. Endocrinology. 136 : 124-131.

Hirsch PF, Voelkel EF, Munson PL, (1964) Thyrocalcitonin hypo-calcemic hypophosphatemic principle of the thyroid gland. Science. 412-413.

Horowitz MC. (1997) Cytokines and estrogen in bone anti-osteoporosis effects. Science. 260 : 626-627.

Hubl W, Fehert T, Ronde W, Dormer G, Taubet HH, Freymann E. (1982). Endocrinology. 79(2) : 165.

Irving JT in Commr. C.L. Bronner F (eds). (1964). Mineral Metabolism. Vol. 2, PT. A. New York Academic Press. Pp. 249-297.

Jane Vincent Corbett, R.N. Ed. D. (1987) Laboratory tests and Diagnostic Procedures with Nursing Diagnoses, Second Edition. Appleton Lange, New Walk.

John R. Lee, M.D., (2006) Osteoporosis treatment during or shortly after menopause "progesterone helps build bone". Natural Hormone Balance and Natura.

Johnson LC. (1964) Morphologic analysis in pathology Kinetics of disease and general biology of bone in Frost HM (ed) Bone Biodynamics. Henry Ford Hospital International Symposium. Boston. Little. Brown. Pp. 543-654.

Joshi UM (cited in http \ www.ELISA.com) (1979) Steroids. 34(1): 35.

Jubiz, Abramown M. Ewing LL, Brickergen, (1972) Calcium kinetics in tissue fluids. Journal of Clinical Endocrinology and Metabolism. 31: 207-213.

Kaplan M. (1972) Renal excretion of phosphate and calcium. The Journal of Medicine. 286 : 200.

Karbach U. (1992) Paracellular calcium transport across the small intestine. American Journal of Clinical Nutrition. 122 : 672-677.

Kasperk C, Fitzsimmons F, Strong D (cited in Williams Textbook of Endocrinology. 2002), (1990) Studies of the mechanism by which androgens enhance mitogenesis and differentiation in bone cells. The Journal of Clinical Endocrinology Metabolism. 71 : 1322-1329.

Kasperk CH. Wergedal JE. Farkey JK. Linkhart TA. Turner RT. Baylink DJ. (1989) Androgens directly stimulate proliferation of bone cells in vitro. Endocrinology. 124 : 1576-1578.

Kenneth S. Saladin, (1998) Anatomy Physiology, The Unity of Form and Function. McGraw Hill, 1st Ed.

Kimberg DV. Schachter D. Schenker H. (1961) Active transport of calcium by intestine effects of dietary calcium. American Journal of Medical Physiology. 2001 : 1256-1262.

Kowarski S. Schacter D. (1973) Vitamin D and adenosine triphophatase dependent on divalent cations in rat intestinal mucosa. The Journal of Clinical Investigation. 52 : 2765-2773.

Krane SM, (1970) Calcium phosphate and magnesium in Rasmussen, H. The International Encyclopedia of Pharmacology and Therapeutics. London. Pergamon Press. P. 19.

Krawitt EL. (1973) Ethanol inhibits intestinal calcium transport in rats. Nature. 243 : 88-89.

Kumar R. (1994) Vitamin D and calcium transport. Kidney Internal.

40: 1177-1189.

Lee JR. (1990) Osteoporosis reversal the role of progesterone. International Clinical Nutrition Reviews. 113, 370-77.

Liewendahl K. (1990) Assessment of thyroid status by laboratory methods. development and perspectives. Scand. The Journal of Clinical Investigation. 50 (suppl 201) 83-92.

Lips P. Asscheman H. Unitewall P. Netelenbos JC. Gooren L. (1989) The effects of cross-gender hormonal treatment on bone metabolism in

male-to-female trans sexuals. The Journal of Bone Mineral Research. 4: 657-662 (Medline).

Lorenzo JA. (1991) The role of cytokines in the regulation of local bone resorption. Critical Reviews of Immunology. 11 : 95-213.

Malava LL, Gupta AK, Aubin JEL. (1995) Leukemia inhibitory factor inhibits osteogenic differentiation in rat calvaria cell cultures. Endocrinology. 136 : 1411-1418.

Malvin RL. Lotspeich WD. (1956) Relation between tubular transport of inorganic phosphate and bicarbonate in the dog. American Journal of Medical Physiology. 187 : 51.

Marcus R. (1991) Estrogens and progestins in the management of primary hyperparathyroidism. The Journal of Bone and Mineral Research. 6 (suppl) S125-S129.

Martin DL. Deluca HF. (1969) Influence of sodium on calcium transport by the rat small intestine. American Journal of Medical Physiology. 216: 1351-1359.

Massey LK. Whiting SJ. (1993) Caffeine. urinary calsium metabolism and gone. American Journal of Clinical Nutrition. 123 : 1611-4.

Massry SG, Coburn JW, Kleeman CR. (1970) Evidence of suppressionof parathyroid gland activity by hypermagnesemia. The Journal of Clinical Investigation. 49 : 1619-1629.

Mendel CM. (1999) The free hormone hypothesis physiologically based mathematical model. Endocrinology Reviews. 10 : 232.

Merton Allen. (July 1999) Associates "Thyroid induced osteoporosis". The Journal of Life Sciences Biotechnology.

Miyaura C, Onoe Y, Inada M. (1997) Increased β-lymphopoiesis by interleukin-7 induces bone loss in mice with intact ovarian function Similarity to estrogen deficiency. Proceedings of the National Academy of Sciences. USA. 94 : 9360-9365.

Morgan KJ (cited by Healing with Nutrition.Com). (1985) Magnesium and calcium dietary intakes of the US population. American Journal of Clinical Nutrition. 4 : 195-206.

Moss DW, Cambell DM. Anagnostonkakarus E , King EJ, (1961) Renal effects of hormonal extracts. Biochemistry Journal. 81 : 440.

Nordin BEC. Need AG. Morris HA. Horowitz M. (1993) The nature and significance of the relationship between urinary sodium and urinary calcium in women. American Journal of Clinical Nutrition. 123: 1615-22.

Nordin BEC In Flesch, H.J.J. Blackwood, and M. Owen (eds), Calcified Tissues 1965, Springer, Berlin. P. 226 (1966).

Onoe Y. Miyaura C. Kaminakayashiki T (cited in Williams Textbook of Endocrinology. 2002). (1999) IL-13 and IL-14 inhibit bone resorption by suppressing cyclooxygenase-2 dependent prostaglandin synthesis in osteoblasts. The Journal of Immunology. 156 : 758-764.

Orloffu ,Steward AF .(1989) Parathyroid hormone-like proteins: Biochemical responses and receptor interaction. Endocrin. Rev. 10 :

476-105.

Oster JR. Perez GO. Vaamode CA. (1978) Relationship between blood pH and potassium and phosphorus during acute metabolic acidosis. American Medical Physiology. 235 : 345-351.

Owens J. Chambers TJ. (1995) Differential regulation of osteoclast formation, interleukin-10 (cytokine synthesis inhibitory factor) suppresses formation of osteoclasts but not macrophages in murine bone marrow cultures. The Journal of Bone and Mineral Research. 10 : S220.

Pacifici R. (1998) Cytokines, estrogen and postmenopausal osteoporosis, the second decade. Endocrinology. 139 : 2659-2661.

Papworth DC. Patrick G. (1970) The kinetics of influx of calcium and strontium into rat intestine in vitro.The Journal of Physiology.210: 999-1020.

Parfitt AM. (1997) Calcium homeostasis In Mundy GR. Martin J (eds) Physiology and Pharmacology of Bone. Berlin Spring Verlag. 1-65.

Parfitt AM In Mundy GR. Martin TJ (eds) . (1993) Physiology and Pharmacology of Bone. Berlin, Springer-Verlag. 1-65.

Peacock M. Robertson WG. Nordin BEC. (1969) Relation between serum and urinary calcium with particular reference to parathyroid activity. Lancet. 1 : 384-386.

Penzes L, Adam A, Boross M. (1973) Effect of lysine in the intestinal absorption of radiocalcium in aging rats. Acta Gerontologica. 3 : 531

Pilbeam CC, Harrison JR, Raisz LG. (1996) Prostaglandins and bone metabolism In Bilesikian JP, Raisz LG, Rodan GA (eds) Principles of Bone Biology. San Diego, Academic Press. Pp. 715-728.

Pointon JJ, Feraneis MJO, Smith R. (1979) Effect of vitamin D deficiency on sacroplasmic reticulum function and troponin C concentration of rabbit skeletal muscle. The Journal of Clinical Sciences. 57 : 257-263.

Posen S. (1967) Some hormonal influence on calcium and phosphorus kineties. Annual International Medical. 67 183.

Potts JT Jr, Deftos LJ, P.K., Rosenberg L.E.,(1974) Parathyroid hormone, thyrocalcitonin, vitamin D, bone and mineral metabolism, in body, P.K. Rosenbergy, L.E Duncan's Diseases of Metabolism, Chapter 20, pp. 1225-1430.

Prestwook KM, Thompson DL, Kenny AM, Seibel MJ, Pilbeam CC, Raisz LG, (1999) Low dose estrogen and calcium have an additive effect on bone resorption in older women. The Journal of Clincal Endocrinology and Metabolismb. 84 : 179-183 (Abstract/Full Text).

Puschett JB (cited in Williams Textbook of Endocrinology, 2002), (1974) Study of the renal tubular interaction of thyrocalcitonin, cyclic adenosine 3'5'-monophosphate 25-hydrocholecalciferol and calcium ion. The Journal of Clinical Investigation. 53 : 756.

Rajkowski KM, Cittanova N, Desfosses B and Jayle MF, (1977) Perhydrocyclopentanephenantherine nucleus and calcium. Steroids. 29-50.

Rasmussen H, Feinblatt J, (1971) The relationship between the actions of vitamin D, parathyroid hormone and Calcitonin calcification. Tissue Research. 6 : 265.

Rasmussen H, Goodman DBP, Friedman N (cited in Williams Textbook of Endocrinology, 2002), (1976) Ionic control of metabolism endocrinology Vol. II, Parathyroid Gland. The American Physiological Society. Pp. 225-264.

Reifenstein EC, Jr, and Albright J, (1974) Mineral metabolism and hormonal influence. The Journal of Clinical Investigation. 26 : 24.

Riggs Bil (cited in Williams Textbook of Endocrinology. 2002). (1973) Quantitative evaluation of treatment for primary osteoprosis. in International Symposium on Clinical Aspects of Metabolic Bone Disease. Amsterdam. Excerpta..

Rosen CJ. (2005) Postmenopausal osteoporosis. The New England Journal of Medicine. 353 : 595-603.

Rosen CJ. Donahue LR. (1998) Insulin-like growth factors and bone. The osteoporosis connection revisiting. Proceedings of Society Experimental Biological Medicine. 219-7.

Schacter D. (1963) Vitamin D and the active transport of calcium by the small intestine. in transfer of calcium and strontium across biological membranes. New York. Academic Press. Pp. 197-210.

Schweikert H. Ruff W. Niederie N. Schafer HE. Keck E. Kruck F. (1980) Testosterone metabolism in human bone. Endocrinology. 94 : 325-329 (Medline).

Shimizu M. Potts JT Jr. Gardella TJ. (2000) Minimization of parathyroid hormone. The Journal of Biological Chemistry. 275 : 21836-21843.

Somjen D. Weisman Y. Harell A. Berger E. Kay AM. (1989) Direct and sex specific stimulation by sex steroid of creatine kinase activity and DNA synthesis in rat bone. Proceedings of the National Academy of Sciences. USA. 86 : 3361-3365.

Sosa M. Lainez P. Arbelo A. Navarro MC. (2000) The effect of 25-dihydroxyvitamin D on the bone mineral metabolism of elderly women with hip fracture. Rheumatology. 39 : 1263-1268.

Statland BE. Nishi HH and Young DS. (1972) Renal tubular handling of calcium and phosphorus. The Journal of Clinical Chemistry. 18 : 1468.

Staum BB (cited in Williams Textbook of Endocrinology. 2002). (1972) Tracer microinjection study of renal tubular phosphate reabsorption in the rat. The Journal of Clinical Investigation. 51 : 2271.

Steven E. Whiting. PhD. (2004) Understanding calcium and estrogen activity (http //www.healingwithnutrition.com).

Storm D. Porter REES. Musgrave K. Vereault D. Patton C. Kessenich C. Mohan S. Chen T. Holick MF. Rosen CJ. (1998) calcium

supplementation prevents seasonal bone loss and changes in Biochemical Markers of Bone Turnover in Elderly New England Women A Randomized Placebo-Controlled Trial. The Journal of Clinical Endocrinology and Metabolism. 83 : 3817-3825.

Takayanagi H, Ogasawara K, Hida S (cited in Williams Textbook of Endocrinology, 2002). (2000) T-cell-mediated regulation of osteoclastogenesis by signaling cross-talk between PANKL and IFN-gamma. Nature New Biological. 408 : 600-605.

Tanzu FS, Navia JM, (1973) Calcitonin inhibition of intestinal phosphate absorption. Nature New Biological. 242 : 221.

Tenenhouse HS, (1997) Cellular and molecular mechanisms of renal phosphate transport. The Journal of Bone and Mineral Research. 12: 159-164.

Tranguada RE, Grant WJ, Peterson CR, (1982) Arch International Medical. 117 : 192-202.

Turner RT, Riggs BL, Spelsberg TC. (1994) Skeletal effects of estrogen. Endocrinology Reviews. 15 : 275-300 (Medline).

Van Os CH, (1987) Transcellular calcium transport in intestinal and renal epithelial cells. Biochemistry and Biophysics Acta. 906 : 195-222.

Vander, Sherman, Luciano. (2001) Human Physiology, The Mechanism of Body Function. 8th ed. McGraw Hill. .

Vanderschueren D, Van Herck E, Suiker AMH, Visser WJ, Schot LPC, Bouillon R, (1992) Bone mineral metabolism in aged male rats. Short and long term effects of androgen deficiency. Endocrinology. 130: 2906-2916.

Vanderschueren, L. Vandenput, S. Boonen, M.K. Lindberg, R. Bouillon and C. Ohlsson. (2004) Androgens and Bone. Endocrinal Reviews. June 1, 25(3): 389-42.

Walling MW, (1977) Intestinal Ca^{2+} and phosphate transport differential responses to vitamin D_3 metabolites. American Journal of Medical Physiology. 2 : E488-E494.

Walser M, (1967) Ion association VI. Interaction between calcium. Magnesium inorganic phosphate citrate and protein in normal human plasma. The Journal of Clinical Investigation. 40 : 723.

Wasserman RH, Fullmer CS. (1983) Calcium transport proteins, calcium absorption and vitamin D. Annual Reviews of Physiology. 45 : 375-390.

Wasserman RH, Taylor AN. (1976) Gastro intestinal absorption of calcium and phosphorus, in Aurbach G.D. (Vol. ed) Greep R.O., Astwood E.V. (Sec ed) Geiger S.R. (bk ed) Handbook of physiology Sec. 7 Endocrinology, Vol. VII Parathyroid Gland Washington D.C. The American Physiological Society. Pp. 137-155.

Wasserman RH, Taylor AN, Lippiells L. (1973) Effect of vitamin C_3 on lanthanum (La^{+3}) translocation Evidence for a shunt Patholgoical Fedrarian Proceedings. 32 : 918.

Williams GA, Hargis GK, Bowser EN (cited in Williams Textbook of Endocrinology, 2002). (1973) Endocrinology. 92 : 687.

Wisdom GB. (1976) Renal adaptation to dietary calcium and phosphorus. Clinical Chemistry. 22(8) 1243-1255.

Woodhead JS, Weeks I. (1985) Circulating thyrotrophin as an index of thyroid function. Annual Clinical Biochemistry. 22 : 455-459.

Yang MG, Thomas JW. (1965) Absorption and secretion of some organic and inorganic constituents throughout and alimentary tract of young calves. The Journal of Nutrition. 87 : 444.

Young DS, Pestaner LC and Gilberman U. (1975) "Effects of Drugs on Clinical Laboratory Tests". Clinical Chemistry. 21 : 3660.

Young MM, Nordin BEC. (1967) The effects of the natural and the artificial menopause on plasma and urinary calcium and phosphorus. Lancet. 2 : 118.

APPINEDEX

APPINDEX

TableA (1) All data of males groups (A1 and B1)

• Group	A_1	B_1	
• Number	15	29	
• Including	Children	Early age	
• Age range (yrs)	Below 15	20-35	
• **Blood parameters**	**Mean ±**	**Mean ±**	**P with A_1**
1) Ca^{++} (mg/dl)	10.8 ± 0.25	9.9 ± 0.27	*
2) P^{---} (mg/dl(	5.6 ± 0.09	4.3 ± 0.3	*
3) Alk. Phosph. (IU/L)	252.8 ± 23.2	83.9 ± 5.01	***
4) Solubility product	60.48 ± 3.2	42.57 ± 2.9	**
5) TSH (μIU/ml)	4.2 ± 0.51	3.7 ± 0.4	NS
6) Total T_3 (ng/ml)	87.6 ± 4.3	92.6 ± 3.13	*
7) Total T_4 (ng/ml)	130.6 ± 11.8	138.95 ± 9.76	*
8) Testosterone (ng/ml)	1.178 ± 0.11	8.31 ± 0.34	***
9) Estradiol (ng/ml)	0.3 ± 0.02	0.47 ± 0.03	*
10) Progesterone (ng/ml)	0.11 ± 0.02	0.25 ± 0.02	*
11) Parathormone (ng/ml)	0.92 ± 0.07	1.2 ± 0.08	NS
• **Urine parameters**			
12) pH	4.9 ± 0.11	5.2 ± 0.17	NS
13) Ca^{++} (mg/dl)	2.8 ± 0.32	3.1 ± 0.19	*
14) P^{---} (mg/dl)	4.7 ± 0.27	5.3 ± 0.3	*

☞ where NS = Non Significant * Significant (P<0.05)

= Highly significant (P<0.01) *=Very highly significant (P<0.001)

TableA (2) All data of males groups (B1 and C1)

• Group	B_1	C_1	
• Number	29	16	
• Including	Early age	Middle age	
• Age range (yrs)	20-35	35-55	
• **Blood parameters**	**Mean ±**	**Mean ±**	**P with B_1**
1) Ca^{++} (mg/dl)	9.9 ± 0.27	10.10 ± 0.33	*
2) P^{---} (mg/dl(	4.3 ± 0.3	4.73 ± 0.17	*
3) Alk. Phosph. (IU/L)	83.9 ± 5.01	72.70 ± 4.3	**
4) Solubility product	42.57 ± 2.9	47.77 ± 1.9	*
5) TSH (μIU/ml)	3.7 ± 0.4	2.9 ± 0.34	**
6) Total T_3 (ng/ml)	92.6 ± 3.13	84.4 ± 3.7	**
7) Total T_4 (ng/ml)	138.95 ± 9.76	127.71 ± 8.10	***
8) Testosterone (ng/ml)	8.31 ± 0.34	6.07 ± 0.41	***
9) Estradiol (ng/ml)	0.47 ± 0.03	0.50 ± 0.01	*
10) Progesterone (ng/ml)	0.25 ± 0.02	0.33 ± 0.03	*
11) Parathormone (ng/ml)	1.2 ± 0.08	1.8 ± 0.09	**
• **Urine parameters**			
12) pH	5.2 ± 0.17	5.7 ± 0.18	NS
13) Ca^{++} (mg/dl)	3.1 ± 0.19	6.7 ± 0.23	***
14) P^{---} (mg/dl)	5.3 ± 0.3	4.3 ± 0.31	**

☞ where NS = Non Significant * Significant (P<0.05)

** = Highly significant (P<0.01)

*** = Very highly significant (P<0.001)

TableA (3) All data of males groups (B1 and D1)

• Group	B_1	D_1	
• Number	29	19	
• Including	Early age	Old age	
• Age range (yrs)	20-35	55-72	
• **Blood parameters**	**Mean ±**	**Mean ±**	**P with B_1**
1) Ca^{++} (mg/dl)	9.9 ± 0.27	10.7 ± 0.41	**
2) P^{---} (mg/dl(	4.3 ± 0.3	5.11 ± 0.12	**
3) Alk. Phosph. (IU/L)	83.9 ± 5.01	65.04 ± 3.7	***
4) Solubility product	42.57 ± 2.9	54.67 ± 2.8	**
5) TSH (μIU/ml)	3.7 ± 0.4	7.8 ± 0.35	**
6) Total T_3 (ng/ml)	92.6 ± 3.13	80.7 ± 2.9	***
7) Total T_4 (ng/ml)	138.95 ± 9.76	119.41 ± 4.9	***
8) Testosterone (ng/ml)	8.31 ± 0.34	4.23 ± 0.27	***
9) Estradiol (ng/ml)	0.47 ± 0.03	0.51 ± 0.02	*
10) Progesterone (ng/ml)	0.25 ± 0.02	0.29 ± 0.014	*
11) Parathormone (ng/ml)	1.2 ± 0.08	2.4 ± 0.09	***
• **Urine parameters**			
12) pH	5.2 ± 0.17	5.5 ± 0.20	NS
13) Ca^{++} (mg/dl)	3.1 ± 0.19	8.3 ± 0.31	***
14) P^{---} (mg/dl)	5.3 ± 0.3	3.8 ± 0.29	***

☞ where NS = Non Significant * Significant (P<0.05)

** = Highly significant (P<0.01)

*** = Very highly significant (P<0.001)

TableA (4) All data of females in groups (A2 and B2)

• Group	A_2	B_2	
• Number	16	98	
• Including	Children	Early age	
• Age range (yrs)	Below 15	20-35	
• **Blood parameters**	**Mean ±**	**Mean ±**	**P with A_1**
1) Ca^{++} (mg/dl)	10.4 ± 0.13	9.8 ± 0.21	*
2) P^{---} (mg/dl(	5.4 ± 0.21	3.8 ± 0.24	*
3) Alk. Phosph. (IU/L)	201.7 ± 19.3	71.7 ± 5.5	***
4) Solubility product	56.16 ± 3.3	34.96 ± 2.8	*
5) TSH (μIU/ml)	4.3 ± 0.41	3.9 ± 0.29	NS
6) Total T_3 (ng/ml)	89.7 ± 5.31	93.2 ± 4.7	NS
7) Total T_4 (ng/ml)	125.3 ± 9.4	131.7 ± 8.1	NS
8) Testosterone (ng/ml)	0.2 ± 0.01	1.3 ± 0.01	***
9) Estradiol (ng/ml)	0.3 ± 0.01	6.2 ± 0.03	***
10) Progesterone (ng/ml)	1.3 ± 0.09	4.8 ± 0.27	***
11) Parathormone (ng/ml)	0.88 ± 0.05	1.3 ± 0.04	*
• **Urine parameters**			
12) pH	5.1 ± 0.01	5.3 ± 0.02	NS
13) Ca^{++} (mg/dl)	3.4 ± 0.21	2.7 ± 0.21	NS
14) P^{---} (mg/dl)	4.5 ± 0.30	6.1 ± 0.17	***

☞ where NS = Non Significant * Significant (P<0.05)

** = Highly significant

*** = Very highly significant

TableA (5) All data of females in groups (B2 and C2)

• Group	B_2	C_2	
• Number	98	49	
• Including	Early age	Middle age	
• Age range (yrs)	20-35	35-55	
• **Blood parameters**	**Mean ±**	**Mean ±**	**P with B_2**
1) Ca^{++} (mg/dl)	9.8 ± 0.21	9.9 ± 0.3	*
2) P^{---} (mg/dl(	3.8 ± 0.24	4.7 ± 0.33	*
3) Alk. Phosph. (IU/L)	71.7 ± 5.5	60.4 ± 5.1	**
4) Solubility product	37.24 ± 2.8	46.53 ± 2.5	*
5) TSH (μIU/ml)	3.9 ± 0.29	3.1 ± 0.20	**
6) Total T_3 (ng/ml)	93.2 ± 4.7	83.3 ± 7.13	**
7) Total T_4 (ng/ml)	131.7 ± 8.1	119.4 ± 7.9	***
8) Testosterone (ng/ml)	1.3 ± 0.01	2.1 ± 0.03	***
9) Estradiol (ng/ml)	6.2 ± 0.03	3.2 ± 0.01	***
10) Progesterone (ng/ml)	4.8 ± 0.27	2.2 ± 0.02	***
11) Parathormone (ng/ml)	1.3 ± 0.04	2.1 ± 0.07	***
• **Urine parameters**			
12) pH	5.3 ± 0.02	5.9 ± 0.03	NS
13) Ca^{++} (mg/dl)	2.7 ± 0.21	5.7 ± 0.23	***
14) P^{---} (mg/dl)	6.1 ± 0.17	5.3 ± 0.17	***

☞ where NS = Non Significant * Significant (P<0.05)

** = Highly significant

*** = Very highly significant

TableA (6) All data of females in groups (B2 and D2)

• Group	B_2	D_2	
• Number	98	18	
• Including	Early age	Old age	
• Age range (yrs)	20-35	55-72	
• **Blood parameters**	**Mean ±**	**Mean ±**	**P with B_2**
1) Ca^{++} (mg/dl)	9.8 ± 0.21	10.3 ± 0.45	**
2) P^{---} (mg/dl(	3.8 ± 0.24	5.43 ± 0.43	***
3) Alk. Phosph. (IU/L)	71.7 ± 5.5	54.4 ± 3.9	***
4) Solubility product	37.24 ± 2.8	55.93 ± 3.6	***
5) TSH (μIU/ml)	3.9 ± 0.29	2.6 ± 0.17	**
6) Total T_3 (ng/ml)	93.2 ± 4.7	76.3 ± 6.7	***
7) Total T_4 (ng/ml)	131.7 ± 8.1	103.4 ± 7.1	***
8) Testosterone (ng/ml)	1.3 ± 0.01	3.2 ± 0.02	***
9) Estradiol (ng/ml)	6.2 ± 0.03	0.77 ± 0.05	***
10) Progesterone (ng/ml)	4.8 ± 0.27	1.2 ± 0.01	***
11) Parathormone (ng/ml)	1.3 ± 0.04	3.2 ± 0.13	***
• **Urine parameters**			
12) pH	5.3 ± 0.02	5.6 ± 0.04	NS
13) Ca^{++} (mg/dl)	2.7 ± 0.21	7.1 ± 0.06	***
14) P^{---} (mg/dl)	6.1 ± 0.17	4.4 ± 0.04	**

☞ where NS = Non Significant * Significant (P<0.05)

** = Highly significant

*** = Very highly significant

TableA (7) Data of group A1 compared to data of group A2

• Group	A_1	A_2
• Number	15	16
• Including	Children	Children
• Age range (yrs)	Below 15	Below 15
• **Blood parameters**	**Mean ± SE**	**Mean ± SE**
1) Ca^{++} (mg/dl)	10.8 ± 0.25	10.4 ± 0.13
2) P^{---} (mg/dl(	5.6 ± 0.09	5.4 ± 0.21
3) Alk. Phosph. (IU/L)	252.8 ± 23.2	261.7 ± 19.3
4) Solubility product	60.48 ± 3.2	56.16 ± 3.3
5) TSH (μIU/ml)	4.2 ± 0.51	4.3 ± 0.41
6) Total T_3 (ng/ml)	87.6 ± 4.3	89.7 ± 5.31
7) Total T_4 (ng/ml)	130.6 ± 11.8	125.3 ± 9.4
8) Testosterone (ng/ml)	1.178 ± 0.11	0.2 ± 0.01
9) Estradiol (ng/ml)	0.3 ± 0.02	0.3 ± 0.01
10) Progesterone (ng/ml)	0.11 ± 0.02	1.3 ± 0.09
11) Parathormone (ng/ml)	0.92 ± 0.07	0.88 ± 0.05
• **Urine parameters**		
12) pH	4.9 ± 0.11	5.1 ± 0.01
13) Ca^{++} (mg/dl)	2.8 ± 0.32	3.4 ± 0.21
14) P^{---} (mg/dl)	4.7 ± 0.27	4.5 ± 0.30

TableA (8) Data of group B1 compared to data of group B2

• Group	B_1	B_2
• Number	29	98
• Including	Early age	Early age
• Age range (yrs)	20-35	20-35
• **Blood parameters** 1) Ca^{++} (mg/dl)	**Mean ± SE**	**Mean ± SE**
	9.9 ± 0.27	9.8 ± 0.21
2) P^{---} (mg/dl(	4.3 ± 0.3	3.8 ± 0.24
3) Alk. Phosph. (IU/L)	83.9 ± 5.01	71.7 ± 5.5
4) Solubility product	42.57 ± 2.9	37.24 ± 2.8
5) TSH (μIU/ml)	3.7 ± 0.4	3.9 ± 0.29
6) Total T_3 (ng/ml)	92.6 ± 3.13	93.2 ± 4.7
7) Total T_4 (ng/ml)	138.95 ± 9.76	131.7 ± 8.1
8) Testosterone (ng/ml)	8.31 ± 0.34	1.3 ± 0.01
9) Estradiol (ng/ml)	0.47 ± 0.03	6.2 ± 0.03
10) Progesterone (ng/ml)	0.25 ± 0.02	4.8 ± 0.27
11) Parathormone (ng/ml)	1.2 ± 0.08	1.3 ± 0.04
• **Urine parameters** 12) pH	5.2 ± 0.17	5.3 ± 0.02
13) Ca^{++} (mg/dl)	3.1 ± 0.19	2.7 ± 0.21
14) P^{---} (mg/dl)	5.3 ± 0.3	6.1 ± 0.17

TableA (9) Data of group C_1 compared to data of group C_2

• Group	C_1	C_2
• Number	16	49
• Including	Middle age	Middle age
• Age range (yrs)	35-55	35-55
• **Blood parameters**	**Mean ± SE**	**Mean ± SE**
1) Ca^{++} (mg/dl)	10.10 ± 0.33	9.9 ± 0.3
2) P^{---} (mg/dl(	4.73 ± 0.17	4.7 ± 0.33
3) Alk. Phosph. (IU/L)	72.70 ± 4.3	60.4 ± 5.1
4) Solubility product	47.77 ± 1.9	46.53 ± 2.5
5) TSH (μIU/ml)	2.9 ± 0.34	3.1 ± 0.20
6) Total T_3 (ng/ml)	84.4 ± 3.7	83.3 ± 7.13
7) Total T_4 (ng/ml)	127.71 ± 8.10	119.4 ± 7.9
8) Testosterone (ng/ml)	6.07 ± 0.41	2.1 ± 0.03
9) Estradiol (ng/ml)	0.50 ± 0.01	3.2 ± 0.01
10) Progesterone (ng/ml)	0.33 ± 0.03	2.2 ± 0.02
11) Parathormone (ng/ml)	1.8 ± 0.09	2.1 ± 0.07
• **Urine parameters**		
12) pH	5.7 ± 0.18	5.9 ± 0.03
13) Ca^{++} (mg/dl)	6.7 ± 0.23	5.7 ± 0.23
14) P^{---} (mg/dl)	4.3 ± 0.31	5.3 ± 0.17

TableA (10) Data of group D1 compared to data of group D2

• Group	D_1	D_2
• Number	14	18
• Including	Old age	Old age
• Age range (yrs)	55-72	55-72
• **Blood parameters**	**Mean ± SE**	**Mean ± SE**
1) Ca^{++} (mg/dl)	10.7 ± 0.41	10.3 ± 0.45
2) P^{---} (mg/dl(	5.11 ± 0.12	5.43 ± 0.43
3) Alk. Phosph. (IU/L)	65.04 ± 3.7	54.4 ± 3.9
4) Solubility product	54.67 ± 2.8	55.93 ± 3.6
5) TSH (μIU/ml)	7.8 ± 0.35	2.6 ± 0.17
6) Total T_3 (ng/ml)	80.7 ± 2.9	76.3 ± 6.7
7) Total T_4 (ng/ml)	119.41 ± 4.9	103.4 ± 7.1
8) Testosterone (ng/ml)	4.23 ± 0.27	3.2 ± 0.02
9) Estradiol (ng/ml)	0.51 ± 0.02	0.77 ± 0.05
10) Progesterone (ng/ml)	0.29 ± 0.014	1.2 ± 0.01
11) Parathormone (ng/ml)	2.4 ± 0.09	3.2 ± 0.13
• **Urine parameters**		
12) pH	5.5 ± 0.20	5.6 ± 0.04
13) Ca^{++} (mg/dl)	8.3 ± 0.31	7.1 ± 0.06
14) P^{---} (mg/dl)	3.8 ± 0.29	4.4 ± 0.04

الملخص العربي

في هذا البحث تمت دراسة تأثير **هرمونات الجنس** على أيض **الكالسيوم والفسفور** وأيضا **أنزيم الفوسفاتيز القلوي** في دم أفراد لا يعانون من أية أمراض أخرى ولكن من أعمار سنية مختلفة .

لقد أجرى هذا البحث على (255) من الأفراد ذات البنية السليمة ظاهريا من المتطوعين والساكنين بشعبيـــة سرت وسبها قسمت هذه الأفراد طبقـــا للعمر إلى (4) مجموعات هي : -

المجموعة الأولى (A) : -

شملت الأفراد تحت سن الخامسة عشر من الجنسين ، وقسمت هذه المجموعة إلى مجموعتين - مجموعة خاصة بالذكور (A 1) ومجموعة خاصـــــــة بالإناث (A 2) ، وهذه المجموعة تحتوي على كميات ضئيلة جدا من الهرمونات الجنسية

المجموعة الثانية (B) : -

وقد شملت الإفراد مابين العشرين عاما والخمسة وثلاثون عاما حيث أن الهرمونات الجنسية في هذه الأفراد أعلى ما يكون إذا قورنت بالمجموعات الأخرى - وقد قسمت هذه المجموعة إلى مجموعتين ، مجموعة خاصة بالذكور (B 1) ومجموعة خاصة بالإناث (B2) ، أتخذت هذه المجموعة كمجموعة قياسية مقارنة بباقي المجموعات حيث أنها المجموعة الوحيدة التي يرتفع فيها هرمونات الجنس .

المجموعة الثالثة (C) : -

حيث شملت هذه المجموعة افرادا تتفاوت أعمارهم ما بين 35 سنة و 55 سنة ، هذه المجموعة تشمل الأفراد في أعمار ما قبل الشيخوخة حيث أن هرمونات الجنس تبدأ في الانتقاص إذا قورنت بالمجموعة الثانية ، وقد قسمت هذه المجموعة إلى مجموعتين مجموعة خاصة بالذكور (C 1) ومجموعة خاصة بالإناث (C 2) .

المجموعة الرابعة (D) : -

وتضم هذه المجموعة مجموعة من الإفراد قد تخطو سن الخامسة والخمسين وحتى سن الثانية والسبعين من الجنسين . هذه المجموعة شملت أناسا قد تضاءلت فيهم الهرمونات الجنسية و بذلك أصبح تأثير قلة هذه الهرمونات واضحا في ارتفاع نسب الكالسيوم والفسفور بالرغم من وجود هشاشة بالعظـــام ، وقد قسمت كباقي المجموعات إلى مجموعتين منفصلتين ، مجموعـــــــة (D1) للذكور ومجموعة أخرى (D2) للأناث .

وقد تم أخذ عينات دم من كل المتطوعين صباحاً لتقدير نسب **الكالسيوم والفوسفور وأنزيم الفوسفاتيز القلوى** وهرمونات الجنس (**التستسترون** فى الذكور **والأستروجين والبروجسترون** فى الأناث) وأيضاً **هرمونات الثيروكسين الثلاثى والرباعي** بالأضافة إلى **هرمون الباراثرمون** . وقد تم أيضاً أخذ عينة بول من كل متطوع لتقدير نسب الكالسيوم والفسفوروتقدير حامضية البول.

ولقد أظهرت النتائج أن هرمونات الجنس (**التستسترون** فى الذكور **والأستروجين والبروجسترون** فى الاناث يقلل من نسب الكالسيوم والفسفور بالدم لأنه يرسب هذين العنصرين فى العظام ، ولذا فقد أرتفعت نسب هذين العنصرين فى المجموعتين اللتين يحتويان على نسب ضئيلة من الهرمونات الجنسية وهما مجموعة **(A)** ومجموعة **(I)** ولكن كان تأثير **الأستروجين والبروجسترون** أكثر فى الأناث عن الذكور ، ولكن أرتفاع نسب الكالسيوم والفسفور فى دم كبار السن الذين يعانون من قلة ونقص فى الهرمونات الجنسية لم يكن نتيجة قلة هذه الهرمونات الجنسية فقط ولكن يعزى أيضاً إلى زيادة **هرمون الباراثورمون** وإلى قلة فى **هرمون الثيروكسين الثلاثى والرباعى** أيضاً الأمر الذى يجعلنا نصل إلى أستنتاج وهو أن هذه الهرمونات قد تكون عاملاً مساعداً فى أحداث هشاشة العظام الملحوظة مع تقدم العمر ولكن ليست هى العامل الأوحد ، فبعض العوامل الأخرى قد تتدخل وهى أرتفاع نسبة **الباراثرمون** وأنخفاض نسبة **الثيروكسين الثلاثى والرباعى** وأيضاً الزيادة فى الوزن والسمنة ، وكثرة أكل المأكولات الدهنية ، وأيضاً إلى قلة ممارسة الرياضة ، وهذه العوامل الثلاث الأخيرة ، قد لوحظت عند دراسة نتائج هذا البحث ، حيث لوحظ تغيراً ملحوظاً وجاداً فى الأفراد كبار السن من الجنسين فى حالات الوزن الزائد وقلة التحرك والحركة وأيضاً نتيجة تناول الوجبات الدهنية أكثر منها فى الناس ذوى الأوزان العادية أوالأكثر حركة أو الذين لا يعتادون أكل المأكولات الدهنية ، وذلك إذا قورنت نتائج كل من الجنسين من كبار السن فى كلتا الحالتين . لذا ننصح كبار السن من الجنسين بتناول كمية اكثر من **الكالسيوم** مع جرعة ما من

التستسترون فى الذكور **والأستروجين والبروجسترون** فى الأناث وذلك لتجنب ولتقليل نسب الفقد من العظام عند تقدم العمر .