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Effect of Khat Chewing and Smoking on Estrogen, Calcium and Lipid profile in Premenopausal Women in Sana'a City

A study Research Submitted to Faculty of Laboratory Medicine
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قال تعالى:

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

﴿إِنَّ الَّذِين آَمَنُوا وَعَمِلُوا الصَّالِحَاتِ

إِنَّا لَا نُضِيعُ أَجْرَ مَنْ أَحْسَنَ عَمَلًا ﴿٣٠﴾﴾

صدق الله العظيم

سورة الكهف- آية (٣٠)

الأهداء

نهدي هذا البحث إلى كل طالب علم يسعى لكسب وتزويد رصيده العلمي والثقافي

إلى مصدر افتخارنا واعتزازنا الى من ان نفتخر ان نرفع أسمائهم عالياً

إلى من قدموا كل ما يملكون في سبيل وصولنا إلى تحقيق كل طموحاتنا وذللوا الصعاب في طريقنا وساعدونا لإكمال مسيرتنا اباءنا الكرام

إلى منبع عزنا وفخرنا من لهن جل حبنا واحترامنا من سهرين من اجل راحتنا وعانين من اجل رفعنا فصارين عنوانا لنجاحنا

الى سعادتنا الدائمة وقلوبنا المضيئة الى من جعل الله الجنة تحت اقدامهن امهاتنا الحبيبات الى الروح الذي سكنت ارواحنا الى اسنادنا وقواتنا

وزهور حياتنا إخواننا واخواتنا الأعزاء

أخيراً كل من شجعنا ووثق بقدراتنا ولهم أثر وذكرى جميلة في حياتنا ولا ينبغي ان ننسئ دكاترتنا ممن كان لهم الدور الأكبر في مساندنا ومدنا

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List of Abbreviation

BaP	Benzo[a] pyrene
BPA	Bisphenol A
Ca	Calcium ion
CNS	Central nervous system
COMT	Catechol-O-methyltransferase
DSMI	Diagnostic statistic manual intravenous
ECF	Extracellular fluid
ERE	Estrogen receptor elements
FSH	Follicle-stimulating hormone
GI	Gastrointestinal
GnRH	Gonadotropin- releasing hormone
GPR30	G protein-coupled
HDL	High- density lipoproteins
HRT	Hormone replacement therapy
LDL	Low- density lipoproteins
LH	Luteinizing hormone
NNN	N`-nitrosonornicotine
PAHS	Polycyclic aromatic hydrocarbons
PCOS	Polycystic ovary syndrome
PTH	Parathyroid hormone
S.D	Standard deviation
SDS	Severity of dependence scale
SHBG	Sex hormone- binding globulin
UK	United Kingdom
USA	United States of America

Abstract

Background: Khat Chewing is a common habit among Yemenis. People chew Khat to get psychstimulation effect in the form of euphoria and excitement resulting from the cathinone contents. Smoking is recognized among the risk factors for osteoporosis. Effect of khat chewing and smoking were previously studied to investigate its effect on hormones and some minerals .Estrogens are steroid Sex hormones play a key role in women's health. High circulating levels of estrogens are associated with an increased risk of breast and endometrial cancer in premenopausal women. Calcium is the most abundant mineral in the body, and is very essential for many activities in the body. Lipid profile refers to the levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG).

Objective: This study is aimed to assess the effects of Khat Chewing and Smoking on Estrogen, Calcium and Lipid profile levels in premenopausal women in Sana'a city with a compared to non- Khat chewer's and non-Smokers .

Methods: Total of one hundred samples were collected from premenopausal women in Sanaa city. Fifty samples were smokers and khat chewers women (Study Group) and 50 samples were non-smokers and a non Khat Chewers women (Control Group), to determine calcium, lipid profile and estrogen levels using ELASA by MAGLUMI for estrogen and Fully-auto chemiluminescence immunoassay (CLIA) analyzer by BECKMAN COULTER for calcium and lipid profile

Result: There were no significantly difference in Estrogen (p-value = 0.216), Calcium (p-value = 0.125), and lipid profile including TC (p-value = 0.262), TG (p-value = 0.062), HDL (p-value = 0.926) while LDL was significantly increased (p-value = 0,0259) in Khat chewers and smokers when compared with non-Khat chewers and smokers.

Conclusion: This study showed that the serum Estrogen non-significant decreased in study group with compared to control ones. Calcium, Total Cholesterol, Triglyceride and High-density lipoprotein (HDL) showed non-significant differences in Khat chewers and smokers' premenopausal woman as compared to non-Khat chewers and Smokers premenopausal woman, however serum Low-density lipoprotein (LDL) was significantly increased in Khat chewers and Smokers premenopausal woman as compared to non-Khat chewers and Smokers premenopausal woman.

Chapter I:

Introduction and Literature Review

1. Introduction

1.1. Khat

Khat is an herbal product consisting of the leaves and shoots of the shrub Catha edulis Forks, a member (genera) of the evergreen celastraceous (moonseed or spindle-tree) family or tribe.(Simmons et al., 2008) Under natural conditions, Khat grows in and on the margins of dry evergreen forest and mist forest. It grows naturally an elevation of 1500-2000 m but is found at altitudes of 1200-2500 m. It is best cultivated at high elevations, with high rainfall in acidic, well-drained clay soils; but can survive long periods of drought. Khat grows in clumps, in beds or rows; often mixed in or interspersed with other crops, such as coffee and conifers, when cultivated.(Balint et al., 1994, Brooke, 1960)

Khat (Catha edulis Forsk) is a perennial plant commonly grown in Yemen, Ethiopia, and other horn African countries.(Girma et al., 2015) Chewers Khat is a social and cultural habit practiced by Yemeni and East African peoples.(Alshagga et al., 2016) The tender, reddish green leaves and the young shoots (Figure 1) are chewed for several hours daily to get the desired effect.(Orlien et al., 2018) Generally, people chew Khat for its stimulant effects on nervous system and reducing fatigue and restore mental and physical activity.(Alsalahi et al., 2016) It has been reported that Ethiopia was the original country of Khat cultivation and then was spread to Somalia, Djibouti, Kenya, and Yemen.(Gebrie et al., 2018) Khat is known by many local names: mirra in Kenya, qat and Khat in Yemen, qaadorjaad in Somalia, and chat in Ethiopia.(Abdeta et al., 2017) It was reported that Khat Chewers prevalence in Yemeni general population is 67 %.(Sutan et al., 2014) A recent study conducted in Saudi Arabia described the high prevalence of Khat Chewers in Jazan region especially by

diabetic patients.(Badedi et al., 2020) The estimated global Khat consumers are about 20 million.

Khat stimulant effect is attributed to its contents of cathinone, cathine, Cath erine, Edline, and ephedrine.(Feyissa et al., 2008) The most active component of K hat is the cathinone, which is considered as a natural amphetamine. It helps chew ers to improve their performance, stay alert, and initially increase their energy.(Cox and Rampes, 2003)

Its central nervous system (CNS) stimulant effect is about 7–10 times highe r than cathine.(Hoffman and Al'Absi, 2010)

Khat physical and psychological hazards were controversially reported by m any authors.(Al-Habori, 2005, Cox and Rampes, 2003, Feyissa et al., 2008, Sawair et al., 2007) The neurotoxic effects of Khat were attributed to the release of dopami ne from presynaptic storage site.(Kalix and Braenden, 1985) Chronic Khat use lead s to a significant depletion of dopamine in several brain areas, particularly the nigro striatal dopamine terminal projections.(Banjaw et al., 2006) Psychiatric effect of K hat includes depression and psychosis among heavy chewers.(Morrish et al., 1999) psychosis precipitation in predisposed persons.(Cox and Rampes, 2003, Odenwal d et al., 2005) and symptom exacerbation in patient with preexisting psychiatric dis orders.(Hassan et al., 2002b) Impaired concentration, insomnia, headache, migraine , mydriasis, impaired motor coordination, and fine tremors are also central nervous system deficits associated with Khat use.(Al-Motarreb et al., 2002) Cox and Ramps , (2003), reported in their review the behavioral effects of Chewers Khat that includ e euphoria, excitability, anxiety, irritability, hyperactivity, restlessness, and insomn ia (24). Khat chewers significantly complain of anorexia, insomnia, late wake-up n ext morning, and low work performance next day.(Hassan et al., 2002b) Anorexia was attributed to both the central amphetamine-like effect and delayed gastric empt

ying(Nasher et al., 1995), while insomnia and delayed bedtime were expected to be due to the cathinone driven release of noradrenergic neurotransmitters.(Giannini et al., 1986)

Physical and psychological dependence of Khat were reported by many aut hors.(Kassim et al., 2010, Patel et al., 2005) Psychological dependence features inc lude chewers urge to get their amount of Khat on the expenses of their vital needs li ke food and mood changes during and after Chewers (Hassan et al., 2002a, Kassim and Croucher, 2006, Nencini et al., 1989, Patel et al., 2005) The Severity of Depen dence Scale (SDS) developed by Gossop *et al.,* (Morrish et al., 1999) was used to e valuate psychological dependence to many abused drugs through collecting data ab out the dependence features.(Gossop et al., 1995) SDS was validated and employed by Kassim and her colleagues to assess the psychological dependence of Khat Che wers among Yemeni population living in UK. Features of physical dependence of K hat Chewers were also reported. Older and habitual Khat chewers showed an increa se in the amount of chewed Khat.(Nencini et al., 1989) Other studies observed that Khat chewers in their long sessions used to refresh the Khat in the mouth.(Sawair e t al., 2007) Some evidences also of withdrawal manifestations were found among K hat chewers, who reported that they continued Khat consumption to avoid unspecifi ed symptoms and to avoid unpleasant feelings and depression.(Alem et al., 1999) O ther withdrawal symptoms reported among Khat quitters were lethargy, feeling slee py, nightmares, feeling hot in the lower extremities, bad temper, and slight tremblin g.(Al-Habori, 2005, Al-Motarreb et al., 2002) When Kassim and her colleagues.(K assim et al., 2013) employed the criteria of dependence defined in the American Ps ychiatric Association Diagnostic Statistic Manual (DSMIV, 1994), they found that 31% of Khat chewers reported dependence syndrome, 13% of them reported an inc rease in Khat Chewers, 19% reported cessation attempts, and 17% reported withdra wal symptoms including depression, increased appetite, and interrupted sleep [52].

1.1.1. Cultivation, Harvesting, and Consumption

In Yemen, Khat is grown in all regions of the central and western highlands, in which most of the Yemeni population is concentrated. In this region, the most common famous types of Yemeni Khat are cultivated.(Zahran et al., 2014) The coastal and desert areas are not suitable climatically for cultivation of Khat. In Ethiopia, Khat is cultivated in almost highlands in the middle altitudes between 1500 and 2100 meters, and the land used to Khat cultivate is greatly increased.(Cochrane and O'Regan, 2016) In Kenya, the cultivation of Khat is also widespread especially in North Eastern Province.(Aden et al., 2006) Regarding Khat gathering, usually in the early morning time, the tender, reddish green leaves are cut together with the twigs by Khat pickers who are usually Khat growers and sellers and collects them in small or large bundles. Then, these bundles are transferred to the market to be sold to consumers on the same day, and if the sale is delayed until the next day, this reduces the quality and price of the Khat due to the conversion of psychoactive cathinone to less active cathine, and this explains the preference of Khat users for fresh leaves and twigs.(Lamina, 2010, Yi et al., 2013) Khat session started usually between 1 and 2 PM daily when the men gather in one place called Majlis or Diwan and start to pick fresh leaves and twigs and chew them on one side to allow juice to be absorbed via the oral mucosa, or through the gastrointestinal tract after swallowing it with well-chewed leaves, adding periodically fresh leaves and twigs to obtain euphoria.(Badedi et al., 2020, Kassim et al., 2015) Normally, each Khat consumer chewed between 20 and 500 grams of Khat daily.

Figure (1): The map of Horn Africa and Yemen.
(https://commons.wikimedia.org/wiki/file:Horn_of_Africa_map.png.

1.1.2. Epidemiology

Widespread in several countries of East Africa and the Arabian Peninsula, o riginated in Ethiopia and spread through Kenya, Somalia, Djibouti, Uganda, Tanz ania, Zimbabwe, Zambia, South Africa, and Yemen.

1.1.3. Chemistry of the Khat

The environment and climate conditions determine the chemical profile of Khat leaves. In the Yemen Arab Republic, about 44 different types of Khat exist originating from different geographic areas of the country.(Kalix, 1987 #65) Its taste varies from one kind to another and depends on the tannic acid content. Khat leaves have an astringent taste and have an aromatic odour. The young leaves are slightly sweet. Many different compounds are found in Khat including alkaloids, terpenoids, flavonoids, sterols, glycosides, tannins, amino acids, vitamins and minerals.(Cox and Rampes, 2003) The phenylalkylamines and the cathedulins are the major alkaloids. The cathedulins are based on a poly-hydroxylated sesquiterpene skeleton and are basically polyesters of euonyminol. Recently, 62 different cathedulins from fresh Khat leaves were characterized.(Kite et al., 2003)

The Khat phenylalkylamines comprise cathinone [S-(–)-cathinone], and the two diastereoisomers cathine [1S,2S-(+)-nor pseudoephedrine or (+)-nor pseudoephedrine] and norephedrine [1R,2S-(–)-norephedrine]. These compounds are structurally related to amphetamine and noradrenaline. The plant contains the (–)-enantiomer of cathinone only; the (+)-enantiomer is not found [56]. Thus, the naturally occurring S- (–)-cathinone has the same absolute configuration as S- (+)-amphetamine. Cathinone is mainly found in the young leaves and shoots. During maturation, cathinone is metabolized to cathine [(+)-nor pseudoephedrine]and (-)-norephedrine. The leaves contain [(+)-norpseudo-ephedrine] and (-)-norephedrine in a ratio of approximately 4:1.(Kalix and Braenden, 1985)

Other phenylalkylamine alkaloids found in Khat leaves are the phenylpent-enylamines merucathinone, pseudomerucathine and merucathine. These seem to contribute less to the stimulant effects of Khat.(Al-Motarreb et al., 2002) and will not be reviewed here. Cathinone is unstable and undergoes decomposition reactions after harvesting and during drying or extraction of the plant material.(Nencini et al., 1989) Decomposition leads to a 'dimer' (3,6-dimethyl-2,5-diphenylpyrazine) and possibly to smaller fragments. Both the dimer and phenylpropanedione have been isolated from Khat extracts.(Narcotics, 1980)

As cathinone is presumably the main psychoactive component of Khat, this explains why fresh leaves are preferred and why Khat is wrapped up in banana leaves to preserve freshness. The phenylalkylamine content of Khat leaves varies within wide limits. Fresh Khat from different origin contained on the average 36 mg cathinone, 120 mg cathine, and 8 mg norephedrine per 100 grams of leaves.(Kalix et al., 1987) Thoennes *et al.,* (2003) found 114 mg cathinone, 83 mg cathine and 44 mg norephedrine in 100grams of Khat leaves confiscated at Frankfurt airport.(Toennes et al., 2003) Wilder *et al.,* (1994) found 102 mg cathinone, 86 mg cathine and 47 mg norephedrine in 100grams of fresh leaves from Kenya confiscated

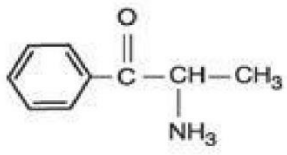
at Geneva Airport [68]. Al-Motarreb *et al.*, (2002) reported higher levels of cathinone in fresh leaves: 78 – 343 mg/100 gram.(Geisshüsler and Brenneisen, 1987)

Khat leaves also contain considerable amounts of tannins (up to 10% in dried material) and flavonoids [58,69].

Chemical Names

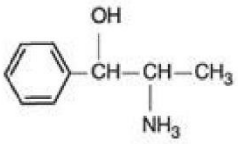
Cathinone: S-(–)-cathinone; S-(–)-α-amino propiophenone; (S)-2-amino-1-phenyl-1-propanone; cathine: 1S,2S-(+)-nor pseudoephedrine; 1S,2S-(+)-phenylpropanolamine; 2-amino-1-phenyl-1-propanol; norephedrine: 1R,2S-(–)-norephedrine; 1R,2S-(–)-phenylpropanolamine; 2-amino-1-phenyl-1-propanol.

Chemical Structures



Cathinone

Figure (2): Chemical structure of cathinone



Cathine, norephedrine

Figure (3): Chemical structure of cathine

Chemical Formula

Cathinone: C9H11NO cathine and norephedrine: C9H13NO D. Molecular Weights: cathinone: 149.2 cathine and norephedrine: 151.2 E. Stereoisomers: Cathinone refers to the naturally occurring S-(–)-cathinone; cathine to the naturally occurring 1S,2S-(+)-norpseudoephedrine; and norephedrine to the naturally occurring 1R,2S- (–)-norephedrine.

Different chemical compounds in Khat

Khat contains many different compounds and therefore Khat Chewers may have many different effects. The major effects include those on the gastro-intestinal system and on the nervous system. Constipation, urine retention and acute cardiovascular effects may be regarded as autonomic (peripheral) nervous system effects; increased alertness, dependence, tolerance and psychiatric symptoms as effects on the central nervous system. As cathinone, and to a lesser extent cathine, are held responsible for the effects of Khat on the nervous system, the effects of the many other constituents of the Khat plant are frequently overlooked. As a consequence, much research has been focused on the pharmacological effects of cathinone and cathine, and much less on the other constituents of Khat.(ECDD, 2006)

1.1.4. Pharmacokinetics

The euphoric effects of Khat start after about 1 hour of Chewers. Blood levels of cathinone start to rise within 1 hour and peak plasma levels are obtained 1.5 – 3.5 hours after the onset of Chewers.(Halket et al., 1995) Maximum plasma levels in this study ranged from 41-141 ng/ml (mean 83 ng/ml) after a 1-hour Chewers dose of 60 g fresh Khat leaves per subject (cathinone: 0.8 – 1 mg/kg body weight). Cathinone was barely detectable at 0.5 h and 7.5 h, and not detectable any more after 24 hours. Six drug-naive male volunteers received a single Khat dose of 54 – 71gram fresh leaves, corresponding to 0.8mg cathinone per kg body weight. Alkaloid-free Khat, prepared by gentle extraction of the alkaloid fraction with 0.1 M hydrochloric acid, was used as the placebo. Chewers was for 1 hour. Maximal plasma concentrations of cathinone (127 ± 53 ng/ml) were attained after 127 ± 30 minutes (values given as the mean± S.D.). The area under the plasma concentration-time curve from 0 to 9 hours was 415 ± 207 ng/ml.hr, and the terminal elimination half-life was 260 ± 102 minutes (63). Comparing these values with those from an

earlier study in which pure S-(–)-cathinone was given to subjects, shows that the time period for reaching the maximal plasma concentration was significantly shorter (72 ± 33 minutes) after administration of pure S- (–)-cathinone. The other values did not differ significantly.(Brenneisen et al., 1990)

In humans, after oral administration of synthesized cathinone (isomers, racemate) 22-52% was recovered in 24 h urine samples mainly as the amino alcohols norephedrine and nor-pseudoephedrine. The main metabolite of S- (–)-cathinone was identified as R, S- (–)-norephedrine and the main metabolite of R- (+)-cathinone as R, R- (–)-nor-pseudoephedrine. Both amino alcohols are apparently formed by a stereospecific keto reduction.Metabolism is rapid and occurs already during first passage through the liver. Only 2% of administered cathinone was found unchanged in the urine.(Brenneisen et al., 1986) The rate of metabolism is close to the rate of absorption, which limits cathinone blood levels reachable by Chewers.(Cox and Rampes, 2003)

In humans, norephedrine and nor-pseudoephedrine are slowly absorbed and excreted almost unchanged in urine.(Maitai and Mugera, 1975) Recently, a pharmacokinetic study was performed with 4 volunteers and a dose of 0.6 gram of Khat leaves per kg body weight, i.e. one fourth of a traditional Khat session dose.(Widler et al., 1994) A two-compartment kinetic model with a two-segment absorption could describe the plasma concentration-time data with a tlag1 (first compartment) of 0.1-0.2 h and a tlag2 (second compartment) of more than 1.2 h. The oral mucosa was seen as the first absorption segment, where the major proportion of cathinone and cathine is absorbed (mean ± S.D.: 59 ± 21% for cathinone; 84 ± 6% for cathine). On the average, peak plasma levels were obtained after 2.3 h for cathinone, 2.6 h for cathine and 2.8 h for norephedrine. Chewers resulted in a high extraction of the alkaloids with only 9.1 ± 4.2% remaining in the leaf residues. The mean ± S.D. Khat dose was 0.63 ± 0.04 mg of cathinone, 0.45 ±

0.03 mg of cathine, and 0.26 ± 0.01 mg of norephedrine per kg of body weight. The mean ± S.D. terminal elimination half-life of cathinone was 1.5 ± 0.8 h and that of cathine 5.2 ± 3.4 h. The apparent volume of the central compartment for cathinone was 2.7 ± 1.6 L/kg and for cathine 0.7 ± 0.4 L/kg. The data obtained for norephedrine could be explained by the metabolism of cathinone into norephedrine.(Toennes et al., 2003)

It has been reported that Khat Chewers delays gastric emptying of a semi-solid meal.(Heymann et al., 1995) It is not known whether this effect is caused by cathinone or by other Khat constituents.

1.1.5. The toxicity of Khat

The effects on the nervous system resemble those of amphetamine with differences being quantitative rather than qualitative.(Cox and Rampes, 2003, Hassan et al., 2002a) The main toxic effects include increased blood pressure, tachycardia, insomnia, anorexia, constipation, general malaise, irritability, migraine and impaired sexual potency in men.(Nencini et al., 1989) Mild depressive reactions have been reported during Khat withdrawal or at the end of a Khat session.(Pantelis et al., 1989) Frequent use of high doses may evoke psychotic reactions. Biochemically, Khat leaves decreased plasma cholesterol, glucose and triglycerides in rabbits,(Al-Habori and Al-Mamary, 2004) and increased plasma alkaline phosphatase and alanine aminotransferase in white rabbits.(Al-Mamary et al., 2002) Histopathological signs of congestion of the central liver veins were observed with acute hepatocellular damage and regeneration. In addition, some kidney lesions were seen with the presence of fat droplets in the upper cortical tubules, acute cellular swelling, hyaline tubules, and acute tubular nephrosis. Spleen was not affected and the histoarchitecture of the testes and cauda epididymis was normal showing, however, increased rate of spermatogenesis. The amount of Khat consumed by the

rabbits cannot be evaluated from the details given. The authors reported that, in general, the activity and the behavior of the animals were observed to be normal.(Al-Mamary et al., 2002)

1.1.6. Effects of Khat

Economic & Social effects of Chewing Khat

The major contemporary problem of the excessive consumption of Khat is the decrease of economic and social productivity through reduction of working hours spent on Chewers Khat as well as overspending for a non - essential commodity on the expense of food with ensuing malnutrition and proneness to disease and last, but not least, loss of needed foreign currency.(Harris, 1981)

This problem is first of today because of the easy access to Khat through air transport and because the economic development of Khat consuming countries cannot tolerate the loss of productivity from the excessive use of Khat.(Harris, 1981)

Health effects of Chewing Khat

The clinical effects of Khat can be fully explained by its main constituent's cathine / one and tannins. They include mydriasis, tachycardia, extra systoles, eleva ted blood pressure, transient facial and conjunctival congestion, headaches, hyperth ermia, increased respiration (through central stimulation, Broncho dilatation, count er regulation of hyperthermia), inhibition of micturition, increased diuresis (from in take of large quantities of fluids together with Khat); cerebral hemorrhage, myocar dial insufficiency, pulmonary edema in predisposed individuals**.**

Increased sympathicotonus is being lived to be, besides psychological Khat effects, the cause of anaphrodisia and spermatorrhoea. Effects on the gastrointestin al tract include stomatitis, gingivitis, esophagitis, gastritis, proneness to buccal and oesophageal epitheliomas and duodenal ulcers. (Harris, 1981)

Constipation is Very common, sometimes with paralytic ileus. Anorexia as a typical amphetamine effect is strong factors in the vicious circle Khat - anorexia - malnutrition - digestive troubles - hunger – its suppression through Khat --- Malnut rition may aggravate inter current disease, e.g., tuberculosis**.**

The reinforcing effects of Khat include euphoria, logorrhoea, and improvem ent of associations, excitement, and insomnia. Toxic psychosis occurs very rarely, i f at all. This and the absence of tolerance are obviously due to the self - limiting pro cess of ingestion. (Harris, 1981)

Symptoms of withdrawal from Khat are rebound phenomena rather than the expression of a true physical dependence. While the qualitative similarity between Khat and amphetamines is evident the quantitative differences are probably of a ph armacokinetic nature yet to be explored. Khat problems hardly existed when its use was acculturated as,e.g., with the Mehru and Isiolo tribes in Kenya where only the e lder herdsmen were permitted to chew Khat. (Harris, 1981)

Tannins contained in qat leaves are responsible for a variety of disorders of the digestive system, such as constipation, gastritis, and loss of appetite. The high r ate of anorexia-like symptoms reported among qat chewers is explained by the app etite-suppressing characteristics of qat, which it has in common with other ampheta mine-type stimulants.

Frequent constipation resulting from qat Chewers has been linked to hemor rhoids, from which 60 percent of qat chewers are reported to suffer. Tannic acid is a lso considered a possible contributing factor to the high level of liver cirrhosis obse rved in Yemen.(Ward and Gatter, 2000)

1.2. Tobacco Smoking

Tobacco Smoking is a significant cause of preventable death and ill health worldwide. Based on current trends, 80% of tobacco-related mortality is predicted to occur in low- and middle-income countries. Reduction/control of tobacco use in these countries is one of the Millennium Development Goals. Ethnicity/culture alongside other factors (e.g., socioeconomic status) contributes to the uptake of tobacco, although determinants of tobacco use are complex. the act of inhaling and exhaling the fumes of burning plant material. A variety of plant materials are smoked, including marijuana and hashish, but the act is most commonly associated with tobacco as smoked in a cigarette, cigar, or pipe. Tobacco contains nicotine, an alkaloid that is addictive and can have both stimulating and tranquilizing psychoactive effects. The Smokers of tobacco, long practiced by American Indians, was introduced to Europe by Christopher Columbus and other explorers. Smokers soon spread to other areas and today is widely practiced around the world despite medical, social, and religious arguments against it.

Smoked forms of tobacco include various kinds of cigarettes (manufactured, hand-rolled, filtered, un-filtered and flavored), cigars and pipes. While cigarette Smokers, particularly manufactured cigarettes, is by far the main form of tobacco smoked globally, in some countries other forms of smoked tobacco are dominant (IARC, 2004a). In India, for example, bidis (made of coarse and uncured tobacco) account for about 60% of smoked tobacco products whereas cigarettes account for 20% (Ray & Gupta, 2009; IIPS, 2010). Water pipes, another form of smoked tobacco known by other various names such as Gaza, hookah, narghile, shisha, hubble-bubble, are commonly smoked in the Eastern Mediterranean region, in some parts of Asia including India, and in North Africa (Asma et al., 2009).

1.2.1. Chemical composition of tobacco

Smoke from cigarettes: - are attributed to nicotine, the principal tobacco alkaloid in smoke. Minor tobacco alkaloids include nornicotine, anatabine and anatabine (Hukkanen et al., 2005). The tobacco alkaloids are not generally considered carcinogenic, but are accompanied by carcinogens in each puff of smoke.

In summary, cigarette smoke is an exceedingly complex mixture which contains over 5300 compounds including multiple toxicants and carcinogens.

1.2.2. Prevalence of tobacco Smokers

Distribution of smokers by WHO region and country:

WHO estimates that in 2009, there was about 1.1 billion adult smokers worldwide, representing nearly a quarter (22%) of the global adult population (WHO, 2011)? A disaggregation by the six WHO regions shows that over a third of smokers worldwide live in WPRO (highly influenced by the People's Republic of China), followed by SEARO, which has around a fifth of the world's smokers (influenced by India and Indonesia). The number of smokers in any country is a function of both the prevalence of Smokers and the size of the population. A further disaggregation of the regions by country shows that a few countries account for a large proportion of tobacco smokers. Ranked in descending order of the number of smokers, the five countries of China, India, United States of America (Chen et al.), Russian Federation and Indonesia account for about 52% of adult smokers in the world, with China and India alone accounting for 40%. Furthermore, nearly two-thirds of the world's smokers live in only ten countries of the world.

1.2.3. Effect Smokers in Cancer in Humans

The available knowledge on the relationship between tobacco Smokers and a variety of human cancers is based primarily on epidemiological evidence. An immense amount of such evidence has been obtained, and only a small proportion can be referred to here. The cancers considered to be causally related to tobacco Smokers in the previous IARC Monograph on tobacco Smokers (IARC, 2004a) included lung, oral cavity, nasal cavity and paranasal sinuses, nasopharynx, oropharynx, hypopharynx, larynx, esophagus (adenocarcinoma and squamous cell carcinoma), upper aerodigestive tract combined, stomach, pancreas, liver, kidney (body and pelvis), ureter, urinary bladder, cervix and myeloid leukemia. In addition, it was concluded that there was evidence suggesting lack of carcinogenicity for cancers of the breast and of the endometrium.

Since 2002, there have been additional cohort and case–control studies on the relationship of tobacco Smokers in different forms to these and other cancers in many countries. A large body of evidence has been obtained from cohort studies with respect to different cancer sites and types of tobacco product. These cohort studies are described briefly.
(available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01), listed by country.

Case–control studies are described in the sections pertaining to cancer sites. More studies are now available from countries and populations that are still at an early stage of the tobacco epidemic. These studies are prone to underestimate the true strengths of the association between tobacco Smokers and any specific cancer as the full effect of duration of Smokers cannot be evaluated.

1.3. Estrogen

The term "estrogen" is derived from estrus or estrum, from the Latin word Oestrus, or the GreekOistros. These terms describe a type of insect, the gadfly, which is indigenous to livestock and cattle breeding areas. Observers noted that during the period of sexual receptivity, cattle behave as though they were being annoyed by a gadfly Estrogen was first introduced into the English language in the 1890s (Arch Gynakol et al; 1900).

Estrogens are a group of hormones that are important for sexual and reproductive development, mainly in women but small amount present in male. They are also referred to as female sex hormones. The term "estrogen" refers to all of the chemically similar hormones in this group (steroids), which are estrone, estradiol (primary in women of reproductive age) and estriol (Mosab Nouraldein et al; 2018)

1.3.1. Site of formation

In women's, estrogens are primarily synthesized in the ovaries by the maturing Graafian follicles, both thecal cells and granulosa cells and incurious luteum. All the three pituitary gonadotropins—FSH, LH and LTH are involved in stimulation of estrogen secretion. Estrogens are also formed in the adrenal gland (the glands on your kidneys), adipose tissue (body fat) and testes in small amounts. The placenta (the organ that allows nutrient - sharing between parent and fetus) secretes estrogen during pregnancy (Allen & Doisy, 1983).

1.3.2. Estrogen Biosynthesis

Androgenic steroids—testosterone and androstenedione—are precursors for the synthesis of estrogens in testes, ovaries, adrenal cortex and placenta. Transformation of the natural C19 steroids, e.g. testosterone and androstenedione to β-estradiol and estrone involves:

Removal of the angular –CH3 group at C10, and Aromatization of ring A. The above is achieved by:

• Enzymes: Aromatase, hydroxylases (mono-oxygenase) and dehydrogenases.

• Hydroxylase: Requires mol O2 and NADPH (Ramakrishnan S, Swamy J.et al; 1995)

Steps

• Cholesterol is converted to pregnenolone and progesterone by the ovarian steroidogenic cells similar to pathway operating in adrenal cortex. Luteal cells also provide certain amount of progesterone.

• Theca internal cells of graafian follicles converts both pregnenolone and progesterone to testosterone and androstenedione, which are the precursors.

• In granulosa cells of the follicle → testosterone is converted to β-estradiol, catalyzed by a microsomal enzyme aromatase, which brings about aromatization of ring A. The enzyme contains a mono-oxygenase as component, it contains Cyt. P450 and requires molecular O2 and NADPH. It brings about three successive hydroxylation's of testosterone and the final hydroxylated product loses C18 nonenzymatically and converted to β-estradiol.

• Androstenedione can be converted to estrone by the enzyme aromatase, with molecular O2 and NADPH.

• β-Estradiol and estrone are interconvertible by the enzyme β-estradiol dehydrogenase.

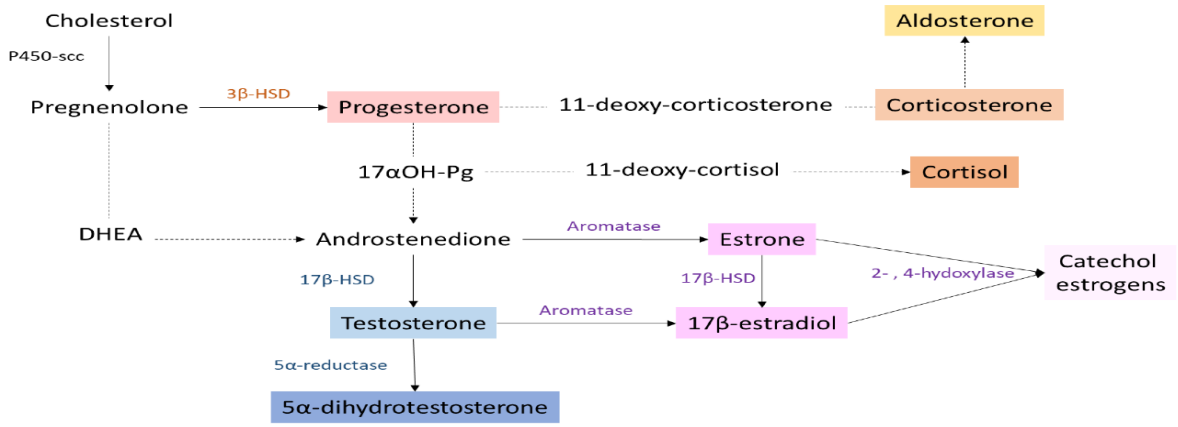
• Estrone is converted to 16-α-OH-estrone by the enzyme 16-α-hydroxylase, also a mono-oxygenase which has Cyt. P450 and requires molecular O2.

Subsequently 16-α-OH-estrone is reduced to form estriol by the enzyme reductase.

• β-Estradiol and estrone can be produced in extra-ovarian tissues, viz. liver, muscles, adipose tissue, etc. from DHEA, androstenedione and testosterone.

• β-Estradiol and estrone are interconvertible in extra-ovarian tissues like liver and placenta, catalyzed by the specific enzyme 17-β-estradiol dehydrogenase (Rawn JD. etal; 1989).

Figure (4): Estrogen biosynthesis pathway.



The estrogen biosynthetic pathway involves the conversion of cholesterol to progestogens, androgens and finally estrogens. The conversion of androgen to estrone (E1) and estradiol (E2) catalyzed by aromatase is the final step for synthesis of estrogen.

1.3.3. Mechanism of Action

Estrogen enters the systemic circulation as a free hormone or protein-bound, either to sex hormone-binding globulin (SHBG) or albumin. Non-protein-bound estrogen has the property to diffuse into cells freely with no regulation. Cellular physiological response to estrogen begins in the cell cytoplasm with estrogen binding to either alpha-estrogen receptor or beta-estrogen receptor. The activated estrogen-estrogen receptor complex (estrogens have been shown to active a G

protein- coupled receptor, GPR30) then crosses into the nucleus of cells to induce DNA transcription by binding to nucleotide sequences known as estrogen response elements (Omar et al.) to enact a physiological response (Sier JH, Thumser AE, Plant NJ; 2017). Estrogen hormone levels in the body are regulated by the negative feedback effect of estrogen on the hypothalamus and pituitary gland. An example of negative feedback can be observed during the menstrual cycle. Estrogen metabolic activity primarily occurs within the liver hepatocytes CYP3A4, and it is excreted from the body in the urine (Hamilton KJ, Hewitt SC, Arao Y, Korach KS; 2017).

The effects of estrogen on various systems of the body are described below:

(A) Breast: Estrogen is responsible for developing mammary gland tissue and parenchymal and stromal changes in breast tissue at puberty in females. Estrogen is also responsible for the development of mammary ducts during puberty and pregnancy, functions to secrete breast milk in postpartum lactation.

(B) Uterus: In the uterus, estrogen helps proliferate endometrial cells in the follicular phase of the menstrual cycle, thickening the endometrial lining in preparation for pregnancy.

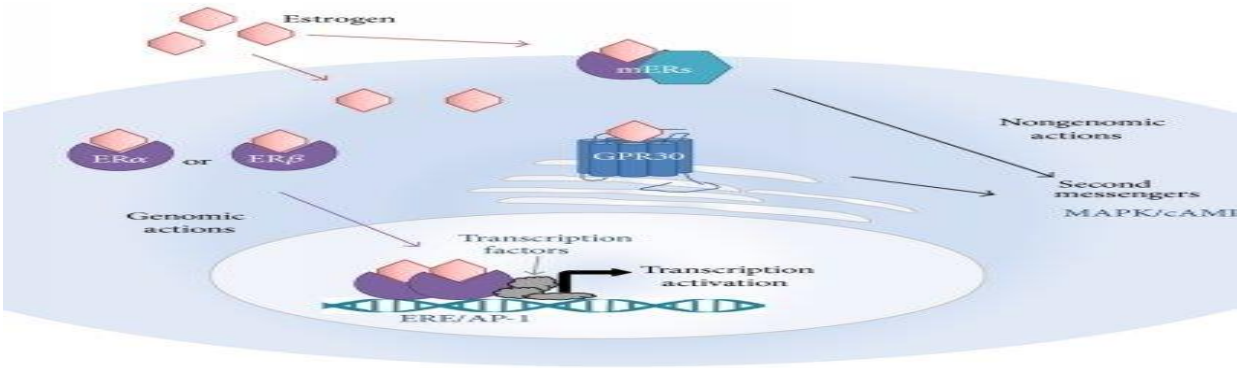
(C) Contraception: Ethinyl estradiol, an ingredient of OCPs, functions to suppress the hypothalamus release of gonadotropin-releasing hormone (GnRH) and pituitary release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in preventing ovulation during the menstrual cycle.

(D) Vagina: Estrogen supports the proliferation of epithelial mucosa cells of the vagina and the vulva. In the absence of estrogen, the vaginal and vulvar mucosal epithelium becomes thin and presents with symptoms of dryness known as vulvovaginal atrophy (Mac Bride MB, Rhodes DJ, Shuster LT; 2010).

(E) Bone: During puberty, estrogen aids in the development of long bones and the fusion of the epiphyseal growth plates (Weise M, De-Levi S, Barnes KM; 2001). Estrogen protects bones by inactivating osteoclast activity, preventing osteoporosis in both estrogen-deficient and postmenopausal women, estrogen is decreased which may cause decalcification and osteoporosis (Klein-Nulend J, van Oers RF, Bakker AD; 2015).

(F) Cardiovascular: Estrogen affects plasma lipids by increasing high-density lipoproteins (HDL) by increased production of apolipoprotein A-1 and decreased hepatic lipase activity, these lead to decrease blood cholesterol and triglyceride levels while decreasing low-density lipoproteins (LDL) by converting hepatic cholesterol to bile acid and total plasma cholesterol and reducing the risk of coronary artery disease with early use in postmenopausal women (Hong MK, Romm PA, Rea1992).

Figure (5): Mechanisms of estrogen action in the cell



1.3.4. Estrogen metabolism

Physiologically, the metabolic conversion of estrogens allows their excretion from the body via urine, feces, and/or bile, along with the production of estrogen analogs, which have been shown to present antiproliferative effects (Tsuchiya et al., 2005). In target cells, there are different pathways capable of metabolizing estradiol

and estrone. Members of the cytochrome P450 superfamily of enzymes (CYP1A1, CYP1B1, and CYP1A2) catalyze hydroxylation of estrone and estradiol at positions C2, C4 and C16. Due to the high expression of these enzymes in the liver, a large proportion of estrogen metabolism occurs in this tissue, although CYP1B1 is also expressed in target tissues such as mammary gland, uterus, kidney, brain, and pituitary gland, where estradiol and estrone can also be metabolized. Estradiol hydroxylation is followed by conversions to 2-hydroxyestrone, 4-hydroxyestrone, 2-hydroxyestradiol, 4-hydroxyestradiol, and 16α-hydroxyestrone, which are also known as catechol estrogens, due to their presence of pharmacological properties of both catecholamines and estrogens. The hydroxylation of estradiol or 16α-hydroxyestrone forms estriol. In addition, catechol estrogens can be methylated via the catechol-O-methyltransferase (COMT) enzyme to methoxy estrogens (Samavat & Kurzer, 2015). These compounds have gained significant attention due to their little estrogenic effects, antiproliferative properties, and ability to control estrogen synthesis (Purohit & Reed, 2002; Purohit et al., 2006). Moreover, catechol estrogens can also be conjugated by estrogen sulfotransferases and UDP-glucanosyltransferase's (Cheng et al., 1998; Garbacz, Jiang, & Xie, 2017). In a conjugation reaction, hormones become water soluble and excreted from the body (Lakhani, Venitz, Figg, & Sparreboom, 2003).

1.3.5. Function of Estrogen

Estrogen regulates important processes in your skeletal, cardiovascular, and central nervous systems that impact your overall health.

Estrogen effects on:

Cholesterol levels, Blood sugar levels, Bone and muscle mass, Circulation and blood flow, Collagen production and moisture in your skin and Brain function, including your ability to focus.

Estrogens are sex steroid hormones, and as such display a broad spectrum of physiological functions. These include regulation of the menstrual cycle and reproduction, bone density, brain function, cholesterol mobilization, development of breast tissue and sexual organs, and control of inflammation (Liang & Shang, 2013).

Physical functions

1) Estrogen is responsible for development of the female body and the secondary sexual characters (breasts, endometrium, regulation of menstrual cycle).
2) Helps decelerate height increase in females during puberty, accelerates burning of body fat and reduces muscle bulk.
3) The stimulates growth of the inner lining of the uterus (endometrium) during the menstrual cycle, increases uterine growth, improves lubrication of the vagina, and thickens the vaginal wall while increasing blood vessels to the skin.

Effects on various biochemical parameters

a) Estrogens reduce bone resorption and increase bone formation.
b) Estrogen help in protein synthesis, increase hepatic production of binding proteins, coagulation factors (factors II, VII, IX, X, plasminogen). Estrogens increase platelet adhesiveness and reduce antithrombin III.
c) Estrogens increase good cholesterol (HDL) and also increase triglycerides. They decrease LDL and promote fat deposition.
d) On fluids and electrolytes estrogens cause salt (sodium) and water retention. In the gastrointestinal tract they reduce bowel motility and increase cholesterol in bile. They also improve lung functions.

Effects on hormones

Estrogens increase cortisol and Sex hormone binding globulin. Estrogens increase melanin and pheomelanin and reduce eumelanin.

1.3.6. Types of estrogens

There are three major forms estrogens that are produced in women, which are estrone (E1), estradiol (E2), and estriol (E3). For women who are in their reproductive years, estradiol is the most active type of estrogen and has the highest levels. The predominant hormones change during pregnancy when estriol levels are higher and during menopause when estrone is the only estrogen that continues to be produced.

Estrone (E1)

Estrone is the primary form of estrogen in males that your body increased makes during in postmenopausal women. Because of that and its connection to an increased risk of cancer it is not included as part of estrogen replacement therapy. Taking estradiol orally can lead to increased levels of estrone as it is metabolized by the liver. Increased levels of estrone in the body are avoided by the use of non-oral methods of estradiol delivery like creams and gels.

Estradiol (E2)

The most commonly prescribed type of estrogen for HRT is estradiol. It is the most potent type of estrogen and the one that is predominant in women during their reproductive years. Replacing estradiol mimics the release of this essential female hormone from the ovaries. Estradiol has the potential to reduce multiple symptoms of menopause including hot flashes, night sweats, and vaginal symptoms. It has also been shown to reduce the risk of osteoporosis and coronary artery disease. Doctors use this form of estrogen as a marker for ovary health.

Estriol (E3)

Is the primary form of estrogen during pregnancy. Estriol is sometimes considered a ³weaker´ estrogen but can be an effective part of HRT, especially when applied locally to treat vaginal symptoms of menopause. Although estriol has been used in Europe for over 60 years, it has failed to gain widespread use in the United States mostly due to the fact that it cannot be patented. (Mosab Nouraldein et al; 2018)

1.3.7. Causes Low Estrogen Levels

The most common cause of low estrogen is age. It's natural for your estrogen levels to fall as you get older.

a) **Low levels unrelated to menopause may be a sign of a condition.**
b) **Age:** Estrogen levels decrease during menopause and premenopausal women. At this point, the primary form of estrogen in your body switches from estradiol (produced primarily in your ovaries) to estrone (produced primarily in body fat).
c) **Eating disorders:** Eating disorders like anorexia and bulimia can deprive your body of the nutrients it needs to keep your hormone levels balanced.
d) **Genetic conditions:** Turner syndrome and Fragile X syndrome both cause low estrogen. **(E) Autoimmune diseases:** Autoimmune diseases that attack your ovaries can prevent them from making enough estrogen.
e) **Primary ovarian insufficiency,** otherwise known as premature menopause. With this condition, your ovaries stop producing eggs before age 40. As a result, your body goes through early menopause. Your periods stop and your estrogen levels decrease.
f) **Treatments impacting your ovaries:** Cancer treatments, like radiation and chemotherapy, may damage your ovaries. The injury may prevent your

ovaries from secreting normal levels of estrogen. Having one or both ovaries removed (oophorectomy) as part of treatment can also cause low estrogen.

g) **Conditions affecting your pituitary gland:** Your pituitary gland secretes hormones that tell your ovaries to start making estrogen. Your body may produce low estrogen levels if your pituitary gland doesn't release enough of these hormones.

h) **Hypothalamic amenorrhea:** If your body is stressed (ex., excessive exercise) and not getting enough nourishment, you can develop hypothalamic amenorrhea. With hypothalamic amenorrhea, your brain doesn't release enough of the hormone that activates estrogen production in your ovaries. As a result, your periods stop entirely. Athletes assigned female at birth are particularly susceptible.

Symptoms of low estrogen

The symptoms associated with low estrogen in your reproductive years overlap with common symptoms associated with menopause and postmenopausal. Your symptoms will depend on what's causing your low estrogen levels.

Signs of low estrogen include

Dry skin, Tender breasts, Weak or brittle bones, Trouble concentrating, Moodiness and irritability, Vaginal dryness oat atrophy. Hot flashes and night sweats. Irregular periods or no periods (amenorrhea), Weight gain, especially in your belly, Headaches before or during your period. Decreased sex drive and painful intercourse (dyspareunia) and Feeling fatigued and having trouble sleeping (insomnia) (Collins Bc, Laakkonen Ek, Lowe DA; 2022).

1.3.8. Causes an Increase in Estrogen

Increase my estrogen naturally resulted foods and supplements that contain ingredients similar to estrogen may help boost your levels.

Speak to your provider before starting any regimen to increase your estrogen.

Also, may rise as a result of:

a) An overproduction of estrogen.
b) Changes in how the body breaks estrogen down.
c) Changes in how the body excretes estrogen.

Any of the above might lead to a hormonal imbalance. In females, estrogen dominance happens when estrogen levels are high compared with the amount of progesterone in the body, which is one of the other main female sex hormones.

1.3.9. Many factors may contribute to estrogen dominance, including

a) **Obesity:** Having excess body weight can result in higher amounts of estrogen due to the fact that fat tissue produces estrone.
b) **Stress:** Stress increases cortisol levels. When cortisol levels remain consistently high, this hormone can deplete the levels of progesterone, which can have a knock-on effect on estrogen.
c) **Alcohol consumption:** Drinking alcohol excessively raises estradiol levels and makes it harder for the body to metabolize estrogen, too.
d) **Dysbiosis:** Intestinal dysbiosis occurs when someone has too many harmful species of bacteria, or not enough beneficial species, in their large intestine. Some types of gut bacteria can reduce how well the body gets rid of excess estrogen, leading to higher levels in the body.

e) **Xenoestrogen exposure:** These chemicals mimic estrogen if they get inside the body. Examples include bisphenol A (BPA) and phthalates, which are present in some plastics. Phthalates are also present in some personal care products, such as soaps and shampoos.
f) **Medications:** Some medications may elevate estrogen or suppress progesterone.
g) **Health conditions:** Some health conditions have an association with or lead to estrogen dominance. These include polycystic ovary syndrome (PCOS), uterine fibroids, endometriosis, and certain cancers. Insulin resistance also increases estrogen levels.

Symptoms of high estrogen levels

Weight gain, especially around the hips and waist, Heavy or light periods, Worse PMS than usual, Fatigue, Fibroids in the uterus, Fibrocystic lumps in the breasts, Low sex drive, Low mood or anxiety andAdditional possible symptoms include Trusted Source: bloating, headaches, disrupted sleep and hair loss.

Complications of high estrogen levels

If a person has consistently high estrogen for an extended period, this increases their risk Trusted Source of:

High blood pressure, Blood clots, Hypocalcemia, which is low calcium, Breast cancer, Cervical cancer and High estrogen may also worsen preexisting conditions, such as asthma or epilepsy.

In 2017 study Trusted Source in South Korea found an association between high levels of free estriol and an increased risk of gestational diabetes in pregnancy. (Stacy Sampson, D.O. et al; 2021)

Table (1): Normal range of estrogen in females.

Follicular phase	15 - 112 pg / mL
Luteal phase	48 - 172 pg / mL
Hormonal contraceptives	15 - 95 pg / mL
Postmenopausal	10 - 60 pg / mL
Preovulatory phase	136 - 251 pg / mL

1.4. Calcium

Calcium is a chemical element with the symbol (Ca) and atomic number 20. It is the most abundant mineral in the body. In combination with phosphorus it forms calcium phosphate, the dense, hard material of the bones and teeth [(1)].

1.4.1. Importance of the calcium

Calcium is an important cation in intracellular and in extracellular fluid and is very essential for many activities in the body such as: Bone and teeth formation, Neuronal activity, Skeletal muscle activity, Cardiac activity, Smooth muscle activity, Secretory activity of the glands, Cell division and growth and Coagulation of blood [(1, 2)].

1.4.2. Body content and normal value of the calcium

body content

In a normal young healthy adult, there is about 1,100 g of calcium in the bo dy. It forms about 1.5% of total body weight. 99% of calcium is present in the bon es and teeth and the rest is present in the plasma.

Normal value of serum calcium

Normal value of serum calcium level ranges between 8.5 and 10.5 mg/dl.(S embulingam and Sembulingam, 2012)

Table (2): Normal value of serum calcium

Age	**Value mg/dl**
Adults	8.5 - 10.5
Children	10 – 12
Newborns	8 – 13

1.4.3. Sources of the calcium

Dietary Source

From foodstuffs: Whole milk (10%), Low fat milk (18%), Cheese (27%), Other dairy products (17%), Vegetables (7%) and Other substances such as: meat, egg, grains, sugar, coffee, tea, chocolate, etc. (21%).(Sembulingam and Sembulingam, 2012)

From Bones:

Besides dietary calcium, blood also gets calcium from bone by resorption.(Sembulingam and Sembulingam, 2012)

1.4.4. Distribution of calcium in the body

The total calcium of the body in adult1100 g. About 99 per cent of it is found in bones. It exists as carbonate or phosphate of calcium. About 0.5 per cent in soft tissue and 0.1 per cent in ECF. The normal level of plasma calcium is 8.5-10.5 mg/dl.(Munjal and Sharm, 2012, Sembulingam and Sembulingam, 2012)

1.4.5. The Calcium in Plasma is present in three forms in plasma

a) Ionized or diffusible calcium: Found freely in plasma and forms about 50% of plasma calcium. It is essential for vital functions such as neuronal activity, muscle contraction, cardiac activity, secretions in the glands, blood coagulation, etc.

b) Non-ionized or non-diffusible calcium: Present in non-ionic form such as calcium bicarbonate. It is about 8% to 10% of plasma calcium.

c) Calcium bound to albumin: Forms about 40% to 42% of plasma calcium.(Munjal and Sharm, 2012, Sembulingam and Sembulingam, 2012)

1.4.6. Daily requirement of the calcium

Table (3): Daily requirement of calcium

Years	**Amount mg**
1 – 3	500
4 – 8	800
9 – 18	1300
19 – 50	1000
51 – above	1200
Pregnant and lactating	1300

1.4.7. Functions of the calcium

a) Calcification of Bones and Teeth: The process of bone formation and teeth formation is known as calcification which is a continuous process for bones.

b) Calcium plays a role in blood coagulation by producing substances for thromboplastic activity of blood.

c) Calcium has a role in neuromuscular transmission.

d) Calcium ions are needed for excitability of nerves.

e) Calcium plays role in muscle contraction.

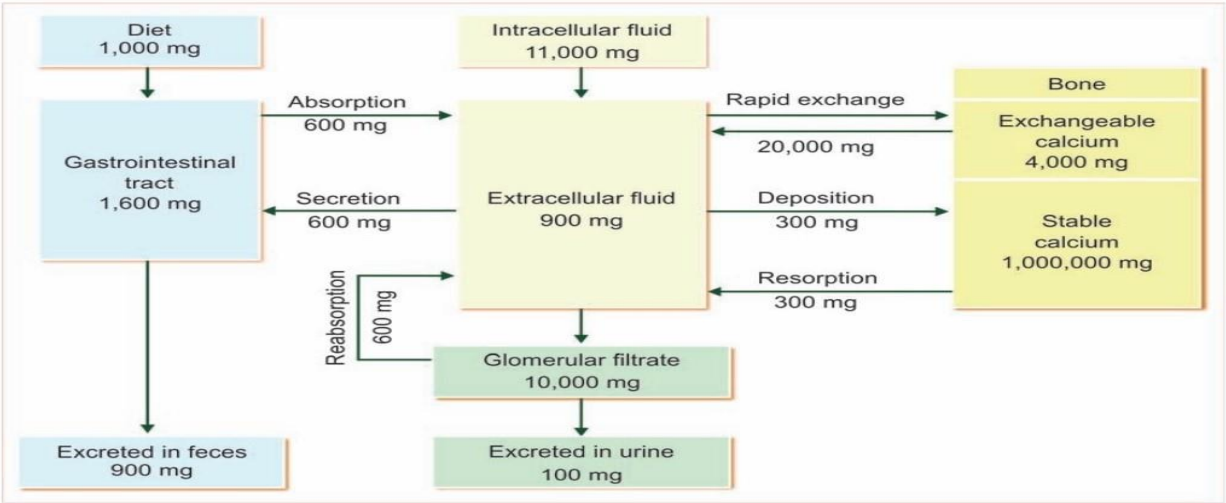
f) Normal excitability of heart is Ca ion dependent.

g) It plays role as secondary or tertiary messenger in hormone action.

h) It plays role in permeability of gap junctions.(Sembulingam and Sembulingam, 2012)

1.4.8. Metabolism to the calcium

Figure (6): Process of calcium metabolism is explained schematically [1]



1.4.9. Absorption of the calcium

Ingested calcium mixes with digestive juice calcium in the proximal small intestine from where it is absorbed by a process, which has an active saturable component and a diffusion component.(Ireland and Fordtran, 1973, Marshall et al., 1976)

At low calcium intakes calcium is mainly absorbed by active (transcellular) transport, but at higher intakes an increasing proportion of calcium is absorbed by simple (paracellular) diffusion. True absorbed calcium is the total calcium absorbed from the calcium pool in the intestines and therefore contains both dietary and digestive juice components. As calcium intake increases, net absorbed calcium also increases, steeply at first but then, as the active transport becomes saturated, more slowly until the slope of absorbed on ingested calcium approaches linearity with an ultimate gradient of about 5–10 percent.(Heaney et al., 1975, Ireland and Fordtran, 1973, Marshall et al., 1976, Nordin and Marshall, 1988)

Factors causing increased Absorption of the calcium

a) Vitamin D: Calcitriol induces the synthesis of the carrier protein (Calbindin) in the intestinal epithelial cells, and so facilitates the absorption of calcium.

b) Parathyroid hormone: It increases calcium transport from the intestinal cells. Acidity: It favors calcium absorption. Amino acids: Lysine and arginine increase calcium absorption.

Factors causing decreased Absorption of the calcium

a) Phytic acid: Hex phosphate of inositol is present in cereals. Fermentation and cooking reduce phytate content.

b) Oxalates: They are present in some leafy vegetables, which cause formation of insoluble calcium oxalates.

c) Malabsorption syndromes: Fatty acid is not absorbed, causing formation of insoluble calcium salt of fatty acid.

d) Phosphate: High phosphate content will cause precipitation as calcium phosphate. The optimum ratio of calcium to phosphorus which allows maximum absorption is 1:2 to 2:1 as present in milk.

1.4.10. Body excretion of the calcium

Urinary calcium is the fraction of the filtered plasma water calcium, which is not reabsorbed in the renal tubules. At a normal glomerular filtration rate of 120 ml/min and ultrafiltrate calcium of 6.4 mg/100 ml (1.60 mmol/l), the filtered load of calcium is about 8 mg/min (0.20 mmol/min) or 11.6 g/day (290 mmol/day). Because the usual 24-hour calcium excretion in developed countries is about 160–200 mg (4–5 mmol), it follows that 98–99 percent of the filtered calcium is usually reabsorbed in the renal tubules. However, calcium excretion is extremely sensitive to changes in filtered load. A decrease in plasma water calcium of only 0.17 mg/100 ml (0.043 mmol/l), which is barely detectable, was sufficient to account for a decrease in urinary calcium of 63 mg (1.51 mmol) when 27 subjects changed from a normal- to a low-calcium diet.

This very sensitive renal response to calcium deprivation combines with the inverse relationship between calcium intake and absorption to stabilize the plasma ionized calcium concentration and to preserve the equilibrium between calcium

entering and leaving the ECF over a wide range of calcium intakes. However, there is always a significant obligatory loss of calcium in the urine (as there is in the faeces), even on a low calcium intake, simply because maintenance of the plasma ionized calcium and, therefore, of the filtered load, prevents total elimination of the calcium from the urine. The lower limit for urinary calcium in developed countries is about 140 mg (3.5 mmol) but depends on protein and salt intakes. From this obligatory minimum, urinary calcium increases on intake with a slope of about 5–10 percent.(Heaney et al., 1999, Marshall and metabolism, 1976, Nordin and Marshall, 1988)

Urinary and endogenous faecal calcium are not the only forms of excreted calcium; losses through skin, hair, and nails need to be taken into account. These are not easily measured, but a combined balance and isotope procedure has yielded estimates of daily insensible calcium losses in the range of 40–80 mg (1–2 mmol), which are unrelated to calcium intake.(Charles et al., 1983, Hasling et al., 1990) The addition of a loss of 60 mg (1.5 mmol) as a constant to urinary calcium loss raises the dietary calcium at which absorbed and excreted calcium reach equilibrium from 520 to 840 mg (13 to 21 mmol).

1.4.11. Regulation of the calcium

Calcium level is remaining within the normal range in blood through its motion between bone, intestine and blood by regulation of three hormones PTH, vitamin D, and calcitonin are known to regulate serum Ca_2 by altering their secretion rate in response to changes in ionized Ca_2.

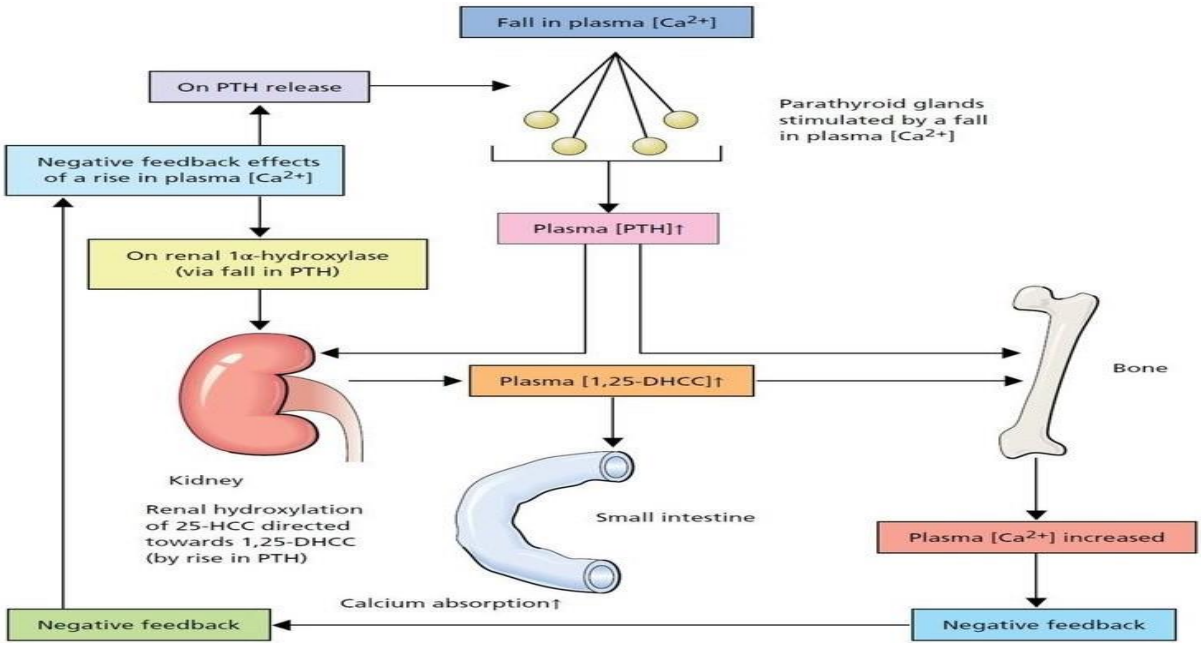
PTH secretion in blood is stimulated by a decrease in ionized $Ca_2$and, conversely, PTH secretion is stopped by an increase in ionized Ca_2. PTH exerts three major effects on both bone and kidney. In the bone, PTH activates a process known as bone resorption, in which activated osteoclasts break down bone and subsequently

release Ca_2into the ECF. In the kidneys, PTH conserves $Ca_2$by increasing tubular reabsorption of Ca_2ions. PTH also stimulates renal production of active vitamin D.

Vitamin D3, a cholecalciferol, is obtained from the diet or exposure of skin to sunlight. Vitamin D3 is then converted in the liver to 25 hydroxycholecalciferols (25-OH-D3), still an inactive form of vitamin D.(MacFadyen et al., 1965) In the kidney, 25-OH-D3 is specifically hydroxylated to form 1,25-dihydroxycholecalciferol (1,25-[OH]2-D3), the biologically active form. This active form of vitamin D increases Ca_2absorption in the intestine and enhances the effect of PTH on bone resorption. Calcitonin, which originates in the medullary cells of the thyroid gland, is secreted when the concentration of Ca_2in blood increases.

Calcitonin exerts its Ca_2- lowering effect by inhibiting the actions of both PTH and vitamin D. Although calcitonin is apparently not secreted during normal regulation of the ionized Ca_2concentration in blood, it is secreted in response to a hypercalcemic stimulus.(MacFadyen et al., 1965) Calcitonin directly cannot affect Ca absorption. Increased calcitonin level inhibits “1-α- hydroxylase” enzyme, thus decreasing synthesis of calcitriol and Ca-absorption, Glucocorticoids: Diminishes intestinal transport of calcium, Calcium is secreted into the gut as a normal constituent of bile and intestinal fluids.

Figure (7): Metabolism of ca^{+2} (101)



1.4.12. Disorders of the calcium

Hypercalcemia

Hypercalcemia is a high Ca^{2+} level in the blood serum with levels greater th an 2.6 mmol/L.

I. Cause of hypercalcemia

Primary hyperparathyroidism (adenoma or glandular hyperplasia), Benign familial hypercalciuria, Malignancy, Multiple myeloma, Increased vitamin D, Thiazide diuretics and Prolonged immobilization.(MacFadyen et al., 1965)

II. Symptoms of hypercalcemia

A mild hypercalcemia: (10.5–12 mg/dl [2.62–3.00 mmol/l]) is often asymptomatic. Moderate or severe Ca2 elevations include neurologic, GI, and renal symptoms. Neurologic symptoms may include mild drowsiness or weakness, depression, lethargy, and coma. GI symptoms may include constipation, nausea,

vomiting, anorexia, and peptic ulcer disease. Hypercalcemia may cause renal symptoms of nephrolithiasis and nephrocalcinosis. Hypercalciuria can result in nephrogenic diabetes insipidus, which causes polyuria that results in hypovolemia, which further aggravates the hypercalcemia. Hypercalcemia can also cause symptoms of digitalis toxicity.

Hypocalcemia

Hypocalcemia is low calcium levels in the blood serum with levels less than 2.1 mmol/l defined as hypocalcemia.

I. Causes of hypocalcemia

Primary hypoparathyroidism (glandular aplasia, destruction, or removal), Hypermagnesemia, Hypoalbuminemia (total calcium only, ionized not affected by)—chronic liver disease, nephrotic syndrome, malnutrition, Acute pancreatitis, Vitamin D deficiency, Renal disease, Rhabdomyolysis and Pseudo-hypoparathyroidism.(MacFadyen et al., 1965)

II. Symptoms of hypocalcemia

Neuromuscular irritability and cardiac irregularities are the primary groups of symptoms that occur with hypocalcemia. Neuromuscular symptoms include paranesthesia, muscle cramps, tetany, and seizures. Cardiac symptoms may include arrhythmia or heart block. Symptoms usually occur with severe hypocalcemia, in which total Ca_2 levels are below 7.5 mg/dl (1.88 mmol/l). Generalized defects in bone mineralization, frequently associated with abnormal calcium or phosphate metabolism, are sometimes grouped together under the term ‘biochemical or metabolic bone diseases. The most common are osteoporosis, rickets and osteocalcin, and Paget’s disease.

Osteoporosis is a systemic skeletal disorder characterized by low bone mass, micro-architectural deterioration of bone tissue leading to bone fragility, and consequent increase in fracture risk. It is the most common reason for a broken bone among the elderly. Bones that commonly break include the vertebrae in the spine, the bones of the forearm, and the hip. Bones may weaken to such a degree that a break may occur with minor stress or spontaneously. After the broken bone heals, the person may have chronic pain and a decreased ability to carry out normal activities.

Osteomalacia is a disease characterized by the softening of the bones caused by impaired bone metabolism primarily due to inadequate levels of available phosphate, calcium, and vitamin D, or because of resorption of calcium. The impairment of bone metabolism causes inadequate bone mineralization. Osteomalacia in children is known as rickets, and because of this, use of the term "Osteomalacia" is often restricted to the milder, adult form of the disease. Signs and symptoms can include diffuse body pains, muscle weakness, and fragility of the bones. In addition to low systemic levels of circulating mineral ions necessary for bone and tooth mineralization, accumulation of mineralization-inhibiting proteins and peptides (such as osteopontin and ASARM peptides) occurs in the extracellular matrix of bones and teeth, likely contributing locally to cause matrix hypo-mineralization (Osteomalacia).(Boukpessi et al., 2010, Salmon et al., 2014) Rickets is a condition that results in weak or soft bones in children.(Syndrome, 2017) Symptoms include bowed legs, stunted growth, bone pain, large forehead, and trouble sleeping. Complications may include bone fractures, muscle spasms, an abnormally curved spine, or intellectual disability. Paget’s disease of bone (commonly known as Paget's disease or, historically, osteitis deformans) is a condition involving cellular remodeling and deformity of one or more bones. The affected bones show signs of dysregulated bone remodeling at the microscopic level,

specifically excessive bone breakdown and subsequent disorganized new bone formation.(Paul Tuck et al., 2017) These structural changes cause the bone to weaken, which may result in deformity, pain, fracture or arthritis of associated joints.(Paul Tuck et al., 2017)

1.5. Lipid profile

Lipids are fatty, wax like molecules found in the human body and other organisms. They serve several different roles in the body, including fueling it, storing energy for the future, sending signals through the body and being a constituent of cell membranes, which hold cells together. Their importance in the biological world is immense. They fill a number of important roles in the cells of all of Earth's organisms. Of the four molecules of life, lipids arguably have the greatest variation in their basic structure and are far more difficult to define than proteins, carbohydrate and nucleic acids. Lipids are essential for all life on Earth. They play many important roles in maintaining the health of an organism.

1.5.1. Classification

Lipids are important fats that serve different roles in the human body. The three main types of lipids are: Triglycerides (also knowns as fats) make up more than 95 percent of lipids in the diet. Triglycerides are lipids you obtain from food sources, e.g. cooking oils, butter, animal fat, avocados, olives, corn, and nuts. Triglycerides provide insulation that keeps you warm while protecting your internal organs with a layer of padding. They also play a role how your body uses vitamins. When you don't burn all the calories you consume, they're converted to triglycerides and stored for future use. Those free fatty acids can then be used by the body to form energy. If you regularly eat more calories than you burn or eat too much food rich in fats, your triglyceride level may become too high and pose a health risk. Phospholipids make up only about 2 percent of dietary lipids. Phospholipids are

derivatives of triglycerides. They're very similar to them but slightly different on a molecular level. Half of each molecule is water-soluble and the other is not, which causes them to react differently than triglycerides. Located on cell membranes, they form double-layered membranes with the water-soluble molecules on the outside of the cell membrane and the water-insoluble molecules in the inside. These lipids are responsible for protecting and insulating cells.

1.5.2. Steroids

Are the least common type of lipid. Cholesterol is perhaps the best well-known steroids. Hormones include the sex hormones estrogen and testosterone, as well as your other hormones like adrenaline, cortisol and progesterone. Cholesterol, the most abundant steroid lipid in the body, is required in every cell in the body. The body gets only a small amount of its cholesterol through food, the body producing most of it. It plays a role in cell repair and the formation of new cells. However, too much cholesterol is a bad thing. When it combines with other compounds in the blood, it can build up as plaque in the arteries. Having a high cholesterol level increases your risk of cardiovascular disease.

1.5.3. Role of Lipids

Energy Production and Storage

While both carbohydrates and lipids provide the fuel to energize your body, carbohydrates are the most readily available source of energy, and lipids function primarily as the body’s backup energy reserves. Fat is energy dense, containing 9 calories per gram, whereas protein and carbohydrate contain only 4 calories per gram. About half of the fuel your body needs when at rest or during everyday activity comes from lipids. If you consume more calories than you need in a day, the excess energy is stored as lipids in adipose cells. In between meals and during exercise your body relies on these fats stores to provide energy.

Insulation and Protection.

Lipids are also used to insulate and protect your body. You have a layer of fat just below your skin that helps to keep your internal body temperature regular despite the external temperature.

Your vital organs e.g. the kidneys, have a layer of fat around them that acts like bubble wrap to protect them from injury. Without this lipid layer, every bump and bruise could hurt your organs.

Cell Wall Structure.

The essential lipids, linolenic acid (the primary dietary omega-6 fatty acid) and linoleic acid, are vital to your health; they cannot be made in your body and must come from your diet. They are used in the production of cell membranes and hormones, as well for maintaining vision and supporting the immune system. These lipids provide structure and support for the walls of every cell in your body. Communication between cells is also dependent upon lipids in your cells' membranes.

Hormone Production

Cholesterol is a type of lipid needed to produce important steroid hormones in your body. Estrogen, testosterone, progesterone and the active form of vitamin D are all formed from cholesterol and are needed to maintain pregnancy, develop sex characteristics and regulate calcium levels in your body. According to the American Heart Association, about 25 percent of the cholesterol in your blood comes from your diet, from animal foods such as egg yolks, cheese and shrimp, and the other 75 percent is formed in your liver and cells.

1.5.4. Digestion and Absorption of Lipids

Lipids in your body are essential for proper digestion and absorption of food and nutrients. Triglycerides are the most common examples of lipid. The insoluble property of lipids makes the digestion and absorption of fats a complicated process. Since they are hydrophobic, fats stick together as a large glob of insoluble mass after reaching the stomach. It is broken down with the help of bile juice, which contains bile salts. These broken molecules are then acted upon by pancreatic lipase, the major fat-absorbing enzymes in the body. Pancreatic lipase breaks down fats into tiny molecules of free fatty acids and monoglycerides, which are small enough for the small intestine to push through into the bloodstream. Lipid digestion, the formation of micelles in the presence of bile salts and passage of micelles and fatty acids through the unstirred (aqueous diffusion) layer.

1.5.5. Lipid Storage

Lipids (e.g. cholesterol, cholesteryl esters and triglycerides) are stored in your body primarily in specialized fat cells called adipocytes, which comprise a specialized fatty tissue called adipose tissue. Stored lipids can be derived from the lipids in your diet or from lipids that your body synthesizes. Adipocytes have an almost unlimited capacity to store lipids in cell organelles called lipid droplets, which can grow to a very large size. Although adipose tissue has essential functions in your body, excessive lipid storage can compromise cell function.

1.5.6. Physiotherapy

As educators the role of lipids in the diet for Athletes should be explained and referred on to a dietician if appropriate. The general public’s view of macronutrients has undergone sweeping changes in recent years. Dietary fats are a key example. Since the anti-fat health education initiatives of the 1980s and early

1990s, certain dietary fats have been increasingly recognized as actually beneficial to health. Athletes, like the mainstream populace, are now getting the message that wise dietary fat (triacylglycerol) choices offer essential fatty acids, blood lipid management, maintained endocrine and immune function, inflammation control, metabolic effects and even potential body composition and performance benefits. The American Heart Association recommends Americans limit their total fat intake to between 25 and 35 percent of total calories. Overindulging in fat from any source increases your calorie intake, putting you at risk for becoming overweight, which is a risk factor for developing diabetes, heart disease, some cancers and high blood pressure. To reduce fat intake, replace high-fat foods with lower fat ones, such as fruits, vegetables, whole-grain breads and cereals. If you eat a diet of 2,000 calories per day, ingest between 44 grams and 77 grams of total fat daily. Food examples of healthy fats e.g. One teaspoon of olive oil contains 4.5 grams of fat of which .621 (g) are saturated fat 3.5 grams are monosaturated fat; large egg contains 4.8grams of fat, and 6.3 grams of protein.

Endurance sport athletes

To maintain good health while training for endurance sports, you need to consume a range of different types of fatty acids which are to be found in saturated fats, mono-unsaturated fats and polyunsaturated fats. Fatty acids are essential components of every cell in your body, forming part of the cell membrane. The brain and nervous system cannot function properly without a healthy cell membrane. Fat is also needed to absorb and transport the fat-soluble vitamins A, D, E and K. The essential Omega-6 fatty acids and Omega-3 fatty acids can only be obtained from food. These types of polyunsaturated fatty acids are necessary to produce hormone like compounds that reduce unnecessary blood clotting, boost immune function and reduce inflammation – all important to an endurance athlete. Exercise-induced damage to muscles triggers increases in strength and endurance.

This damage also leads to inflammation in the muscles. When muscles are inflamed, they are sore, also losing strength and range of motion. Omega-3 fatty acids especially are needed to regulate the level of inflammation in your body. A diet low in omega-3 fats, while high in the more common omega-6 fats, can bias your body towards inflammation, impairing exercise recovery. You can get high amounts of omega-3 fats from fatty fish, algae, and several high-fat plant foods. eg salmon, oysters, sardines, linseeds.

1.5.7. A lipid disorders

Will typically increase levels of LDLs, triglycerides, or both. HDLs are known as good cholesterol because they help to transport bad cholesterol out of the body.

A buildup of LDLs and triglycerides can cause fatty materials to accumulate in the body's tissues, including in the arteries. This can have serious consequences for cardiovascular health and increase the risk of problems such as heart disease. The American Academy for Family Physicians say 1 in 3 adults in the United States have high levels of LDL cholesterol. This is why healthcare professionals advise people to cut down on fatty foods and increase their HDL levels to help remove the bad cholesterol.

Symptoms of lipid disorder

It is important to point out that, most of the time, a person will have no symptoms of a lipid disorder until they experience a significant health problem, such as a stroke or heart attack. However, the following symptoms have been occasionally observed in some people with very high lipid levels: yellowish, fatty bumps or yellow creases on the skin, formed by an accumulation of fatty deposits around tendons and joints (xanthomas)white arcs around the cornea of the eye (arcus

senilis), which sometimes occur in younger people with high cholesterol raised, yellow lumps at the inner corners of the eyes (xanthelasma).

Causes of lipid disorder

A range of factors can cause lipid disorders, including genetics, lifestyle, and other underlying health conditions.

I. **Genetic factors:** Many lipid disorders are inherited. Familial hyper-cholesterolemia (FH) for example, is when high cholesterol runs in a family. FH affects an estimated 1 in 200–500 people worldwide but is more common in people of French Canadian, Ashkenazi Jewish, Lebanese, or Afrikaner (a South African ethnic group) descent. Knowing their family history of cholesterol and heart disease may help people decide if they want to get a blood test to check if they have high cholesterol.

II. **Diet and lifestyle factors:** Foods that are high in saturated fats can also cause high blood cholesterol and high levels of triglycerides. Foods that contain saturated fats include: dairy products, such as cheese, cream, and butter sugary treats, such as cakes, cookies, and ice cream fatty or cured meats, such as sausages, bacon, and salami foods containing coconut oil, palm oil, or lard Foods that contain trans fats are particularly dangerous, as they raise bad cholesterol and lower good cholesterol. Foods that contain trans fats include: baked goods such as cookies, pies, and pastries fried, fatty foods, such as fried chicken, French fries, and doughnuts shortening, certain margarines, and vegetable oils non-dairy coffee creamers Other dietary and lifestyle factors that can elevate cholesterol include: Smokers or exposure to tobacco smoke being physically inactive having overweight or obesity drinking too much alcohol taking certain medications, such as diuretics Medical conditions Underlying health conditions may increase

cholesterol levels, including: diabetes, hypothyroidism, kidney disease and liver disease.

Diagnosis of lipid disorder

A doctor can run a blood test called a lipid profile or lipid panel to initially diagnose a lipid disorder. This measures levels of total cholesterol, LDLs, HDLs, and triglycerides, among other things. If blood test results show elevated cholesterol, a doctor may order other tests to rule out underlying problems that may be causing it, such as thyroid or liver tests. Occasionally, if a doctor suspects a patient needs intensive treatment, they may order advanced lipid testing. This checks the concentration of lipoproteins in the blood and helps to predict the risk of cardiovascular disease more accurately.

1.2. Objectives of the Study

1.2.1. General Objective

Assessment the Effects of Khat Chewing and Smoking on Estrogen, Calcium and Lipid profile levels in premenopausal women with compare control premenopausal women.

1.2.2. Specific Objectives

1. To determine the levels of Estrogen, Calcium and Lipid profile among Khat Chewers and Smokers premenopausal Yemeni women in Sana'a City.
2. To detect the effects of Khat Chewing and Smoking on Estrogen, Calcium and Lipid profile levels.
3. To compare the percentage of Estrogens, Calcium and Lipid profile between Khat Chewers and Smokers premenopausal women and control premenopausal women.
4. To identify the risk factors of Khat Chewing and Smoking in premenopausal wamen.

Chapter II:

Materials and Methods

2.1. Study period

This study was conducted from August /2022/ to October /2022.

2.2. Study area and population

We have determined to the study in Sana'a city (capital of Yemen), as it is the largest in terms of population, located in the center of the country.

2.3. Study subject

Individual sample was chosen to be the healthy women (adult female), age of them range from 30 to 45 yrs., and, we divided them into two groups, first one is the study group which include women who are Khat chewer's and Smokers and the second is control group in which women are non Khat chewer's and smokers.

2.10. Sample size

A total one hundred samples were collected from premenopausal women in Sanaa city. Fifty samples were the khat chewers and smokers women (Study Group) other 50 samples were non- khat chewers Khat and non-smokers women (Control Group), to determine calcium, lipid profile and estrogen levels using ELASA by MAGLUMI for estrogen and Fully-auto chemiluminescence immunoassay (CLIA) analyzer by BECKMAN COULTER for calcium and lipid profile.

2.5. Type of study

- Case control study.

2.6. Data collection

Questions were prepared within a short questionnaire and distributed questionnaires to each individual sample face-to-face interview, we took samples from them.

2.7. Materials and Method

- Syringes, Alcohol pads, Plain tap tubes, Plastic tubes, Eppendorf tubes, Pasture pipettes, Micropipettes, Tips, Sharp container, Gloves, Centrifuge and Ice packs.
- Questioner were used to get the necessary information data sample.

2.8. Specimen collection

Without applying tourniquet, blood was collected from vein by vein puncture then collected into -100 -plan tap tubes free from anticoagulant separated.

2.9. Transportation of serum samples

Serum samples are immediately transported with ice packs into laboratories (Natio nal Center of Public Health Laboratories) Which is located in AL-zyraah' Street of Sana'a city, for investigation of calcium, lipid profile and estrogen levels in each 10 0 sample.

2.10. Sample measurement

Estradiol measurement

Principal Competitive immunoluminometric assay: Use an anti-E2 monoclonal antibody to label ABEI and use a purified E2 antigen to label FITC. Sample, Calibrator or Control, ABEI Label and Displacing reagent are added first and incubate at 37 ℃ for 10 min. Then add FITC Label and Nano magnetic microbeads coated with anti-

FITC and incubated at 37℃ for the second time, forming antibody-antigen complexes; After sediment in a magnetic field, decant the supernatant, then cycle wash for 1 time. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of E2 present in samples.

Calcium and lipid profile measurement

Principal In principle, a calcium ion selective electrode measures un-bound free calcium ions in solution. Total calcium can only be calculated from free calcium when the molar ratio between free and total calcium concentrations is constant. This constant molar ratio is achieved by the buffered solution which contains strong calcium complexing agents. A precise volume of sample (40 microliters) is mixed with the buffered solution. The ratio used is one-part sample to 33 parts buffered solution. The high molar strength buffer is used to establish a constant activity coefficient for calcium ions, calibrating the electrode to concentration values.

2.11.Procedure

2.11.1. Procedure of Estradiol

To ensure proper test performance, strictly adhere to the operating instructions of the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI. Each test parameter is identified via a RFID tag on the Reagent Integral. For further information please refer to the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI Operating Instructions.

Table (4): The procedure of estradiol.

80µl +40µl +20µl	Sample, calibrator ABEI Label Displacing Reagent
10 min	Incubation
+40µl +20µl	FITC Label Nano magnetic microbeads
10 min	Incubation
400µl	Cycle washing
3 s	Measurement

Dilution

Samples with concentrations above the measuring range can be diluted. After manual dilution, multiply the result by the dilution factor. After dilution by the analyzers, the analyzer software automatically takes the dilution into account when calculating the sample concentration. Availability of sample dilution by analyzer please refers to the MAGLUMI analyzer user software program. Dilution settings please follow MALGUMI analyzer operating instructions.

2.11.2. Procedure of Calcium and lipid profile

To ensure proper test performance, strictly adhere to the operating instructions of the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI. Each test parameter is identified via a RFID tag on the Reagent Integral. For further information please refer to the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI Operating Instructions.

Table (5): The procedures of calcium and lipid profile.

80µl +40µl +20µl	Sample, calibrator ABEI Label Displacing Reagent
10 min	Incubation
+40µl +20µl	FITC Label Nano magnetic microbeads
10 min	Incubation
400µl	Cycle washing
3s	Measurement

Chapter III:

Results And Discussion

3.1. The Results.

This study included one hundred samples collected from premenopausal women in Sanaa city. 50 samples were selected from women who smoke and chew Khat (Study Group) and 50 samples were selected from women who non-smoke and non-chew Khat (control Group).

Table (6): Comparative analysis of Estrogen in Smokers and chewers Khat women and non-Smokers and non-chewers Khat women.

parameters	**Study Group (n=50)**	**Control Group (n=50)**	***p*.Value**
Estrogen	84.1908 ±67.30	118.18±99.593	0.216

Value in this table were expressed in Mean± SD (mg/dl). *p*-value non- significant ($\boldsymbol{p} \leq 0.05$).

Table (7): Comparative analysis of calcium in Smokers and chewers Khat women and non-Smokers and non-chewers Khat women.

parameters	**Study Group (n=50)**	**Control Group (n=50)**	***p*.Value**
Ca (mg/dl)	1.11±0.146	1.160±0.1005	0.125

Value in this table were expressed in Mean± SD (mg/dl).. *p*-value non- significant ($\boldsymbol{p} \leq 0.05$).

Table (8): Comparative analysis of Total cholesterol in Smokers and chewers Khat women and non-Smokers and non-chewers Khat women.

parameters	**Study Group (n=50)**	**Control Group (n=50)**	***p*.Value**
T.C (mg/dl)	175±36	169±53	0.262

Value in this table were expressed in Mean± SD (mg/dl).. *p*-value non- significant ($\boldsymbol{p} \leq 0.05$).

Table (9): Comparative analysis of Triglyceride in Smokers and chewers Khat women and non-Smokers and non-chewers Khat women.

parameters	**Study Group (n=50)**	**Control Group (n=50)**	***p*.Value**
TG (mg/dl)	151±74	108±61.3	0.062

Value in this table were expressed in Mean± SD (mg/dl). *p*-value non- significant ($\boldsymbol{p} \leq 0.05$).

Table (10): Comparative analysis of High-density lipoprotein in Smokers and chewers Khat women and non-Smokers and non-chewers Khat women.

Parameters	**Study Group (n=50)**	**Control Group (n=50)**	***p*.Value**
HDL (mg/dl)	84.2±67.30	118.18±99.59	0.926

Value in this table were expressed in Mean± SD (mg/dl). *p*-value non- significant ($\boldsymbol{p} \leq 0.05$).

Table (11): Comparative analysis of low-density lipoprotein in Smokers and chewers Khat women and non-smokers and non-chewers Khat women.

Parameters	**Study Group (n=50)**	**Control Group (n=50)**	***p*.Value**
LDL (mg/dl)	118±27	102±32	0.0259

Value in this table were expressed in Mean± SD (mg/dl). *p*-value significant ($\boldsymbol{p} \leq 0.05$).

3.2. Discussion:

Smoking and chawing Khat had become a daily activity in people's life in Yemen. They are one of the most important causes that lead to a large number of health disorders for individuals. Previous investigations of the Khat effects have spanned various physiological and metabolic effects.

There are several studies dealing with the effects of Khat on different part of body.

This study showed a significant increase of LDL level in study group with compared to control group which go n agreement with a more recent study on healthy university Yemeni male students (n = 360) in which they noted the same observation of increased serum levels of TC and LDL-C in Khat chewers (n=283) than non-Khat chewers (n =77).

Furthermore, total cholesterol, triglyceride, calcium, HDL and Estrogen levels showed non-significantly differences in women who smoke and chew Khat with respect to healthy control in our study.

Our study demonstrated THAT non-significant increase of TC in Smokers and Khat Chewers women with compared to control. In one recent Ethiopian cross-sectional-based study (n = 9800), it has been found a significantly low median of TC and LDL-C in Khat consumers compared with non-Khat consumers. The study of Masoud et al. which included only women, demonstrated similar reducing effects of Khat on TC and LDL-C in female Khat users comparing with non-Khat users.

HDL is referred to as good cholesterol because of its role in the transport of excess cholesterol from peripheral tissues to the liver, preventing atherosclerotic plague formation. The increase of HDL level reduces the risk of CVD. In our study there was nonsignificant decrease of HDL in Smokers and Khat Chewers women

comparing with control. A study established in 2009 reported significant decreased in HDL-C level in Khat chewers compared to non-Khat chewers (24:72 ± 4:48mg/dl versus 33:34 ± 1:83mg/dl).

The effects of Chewers Khat and Smokers on triglycerides, the result of studies in TG are different. In one Yemeni study in 2003 it has been shown increasing levels of fasting TG in Khat chewers than nonKhat chewers (92:77 ± 49:26 versus 85:15 ± 36:73mg/dl). Additionally, Ali et al. demonstrated higher serum TG levels in ACS Khat user patients than nonKhat user patients (288:7 ± 525:0mg/dl vs. 210:0 ± 787:5mg/dl; P < 0:003). Neki NS, during his study on the association between the lipid profile and chronic Smokers, found very low levels of HDL, with an increase in the levels of TC, LDL, VLDC and TG in smokers. This agreed with the findings of our study. Our study demonstrated nonsignificant increase of TG in Smokers and Khat Chewers women comparing with control.

Previous studies on Estrogen often showed decrease in its level. A study in KSA (Saeed et 2012) showed that the level of E2 in serum found to be significantly higher in Khat chewers than in normal control. The study carried out by Ruan and Mueck revealed that duration and intensity of Smoking considerably affect both endogenous and exogenous E2 levels and may cause a decrease or inactivation of this hormone.33. In our study, there was nonsignificant decrease of estrogen in smokers and khat chewers women compared with control.

In addition to, our study determined that there was no difference between the Smokers and Khat Chewers women and control. This may be due to presence of the calcium in Khat tree. Bafghi et al. stated that salivary calcium levels in smokers were lower, although nonsignificant compared to those in nonsmokers.

Chapter IV:

Conclusions and Recommendations

4.1. Conclusions.

Our study demonstrated the effects of Khat chewing and Smoking on calcium, estrogen and lipid profile levels among Khat Chewers and smokers' premenopausal women, and determination of calcium, lipid profile and estrogen levels in non Khat Chewers and Smokers premenopausal women, we compared between them. In this study conducted a case control study. These observations suggest that there was no significant difference in total cholesterol, triglyceride, calcium and estrogen levels in Khat Chewers and Smokers with respect to non-Khat Chewers and smokers' premenopausal women. Serum calcium in both groups was in normal range, also our study shown that the Khat Chewers and Smokers have significant increase in LDL comparing with control group.

4.2. Recommendations.

Since Khat Chewers is wide spread in Yemen, the following actions are recommended:

1. Increase public awareness of the potential health hazards of Khat Chewers
2. Support scientific research on Khat in different institutions and universities to explore the different effects of Khat in public health.
3. Recommend another study on the use of pesticides in the cultivation of Khat in view of their potentially harmful effects on human health.
4. Implementation of other studies with large size of sample and Implementation tests for low-density lipoprotein to determine the case of rise.
5. Follow up premenopausal women over 35 years by specialists regularly.

Chapter V:

Reference

5.1.Reference

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