Potential Role of Arginine, Glutamine and Taurine in Ameliorating Osteoporotic Biomarkers in Ovariectomized Rats

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Abstract

The main purpose of the present study was to evaluate the role of some amino acids namely L-arginine, L-glutamine and taurine in the management of osteoporosis in ovariectomized (OVX) rats. The current study included six groups of female rats which were classified as gonad intact control group and five ovariectomized groups: one untreated group served as ovariectomized control group another ovariectomized group orally administered with 10% lactose, three ovariectomized groups orally administered with each amino acid dissolved in 10% lactose. The treatment was started after 3 months of ovariectomy and continued for other 3 months. Serum parathyroid hormone (PTH), 1, 25 dihydroxyvitamine D_3 levels were determined. Insulin like growth factor-1 (IGF-1) and transforming growth factor-β (TGF-β) levels were also estimated. Bone mineral density (BMD) and bone mineral content (BMC) of right femur bone of each rat were measured using DEXA technique. Also, histological investigation of the bone sections of left femur of each rat was carried out. The obtained data revealed that ovariectomy decreased serum 1, 25 $(OH)_2 D_3$, IGF-1 and TGF-β levels whereas, it increased serum PTH level. DEXA results revealed that ovariectomy decreased BMD and BMC of the proximal, distal and mid areas of rat femur bone. These results were well documented by bone histological examination. The selected amino acids could improve all the studied bone biochemical markers significantly. DEXA results also showed that treatment with these amino acids could increase both BMD and BMC of rat femur bone in most areas. The photomicrographs of femur bone sections of rats treated with the selected amino acids supported the present improvement in bone biomarkers. In conclusion, each of the selected amino acids exhibited antiosteoporotic effects due to the anabolic and/or antiresorptive activity. These encouraging results provide new concepts for the development of effective opportunities in the treatment of primary osteoporosis. "[Report and Opinion. 2009;1(6):24-35].(ISSN:1553-9873)".

Key words: Osteoporosis, L-arginine, L-glutamine, taurine, lactose, bone biomarkers, bone mineralization.

Introduction

Osteoporosis is a global health problem that will take an increasing significance as people live longer and the world's population continues to increase in number, thus the management of osteoporosis and its complications is an socioeconomic priority **(Kevin, 2007)** Osteoporosis is defined as decreased bone strength and increased susceptibility to fractures (**Preisinger , 2009)**. It is also defined as progressive systemic skeletal disease characterized by low bone mass with a consequent increase in bone fragility and susceptibility to fracture. **(Katherine et al., 2007).** So, there is an urgent need to develop and implement alternative nutritional approaches and policies for treatment of osteoporosis **(Kevin, 2007).** This idea comes from the fact that protein under nutrition is known to play an important role in the pathogenesis of osteoporotic fracture. The mechanisms underlying the bone loss in protein under nutrition appeared to be related to an uncoupling between increased bone resorption and bone formation. This was associated with decreased plasma insulin-like growth factor-1 (IGF-1) level, with anoestrus and decreased muscle mass. Nutritional intervention with amino acid supplements can increase bone mineral mass, bone strength and muscle mass in osteoporotic subjects **(Ammann et al., 2000).**

Amino acids are the building blocks of protein. Essential amino acids (EAA) can
Modulate the growth and the differentiation of osteoblasts cultured in vivo, confirming the relationship between osteoporotic hip fracture and inadequate protein intake. Amino acids have mainly enhanced cell growth and alkaline phosphatase activity, and, to a lower degree, collagen synthesis **(Conconi et al., 2001).**

Amino acids supplement increased bone mineral mass and strength in ovariectomized protein-deprived rats. This was associated with stimulated bone formation and reduced bone resorption, with an increment of plasma isulin-like growth factor (IGF-1) and limb muscle mass weight **(Ammann et al., 2000).**

L-arginine represents a key building block to repair damage tissue and bone. Athletes have also

found L-arginine to be beneficial for muscle recovery and growth hormone (GH) release from pituitary gland. Oral administration of L-arginine in pharmacological doses induces growth hormone and insulin –like growth factor-1 responses and stimulates nitric oxide synthesis **(Baecker et al., 2005)**. Growth hormone and insulin –like growth factor-1 is important mediators of bone turnover and osteoblastic bone formation, while nitric oxide is a potent inhibitor of osteoclastic bone resorption. Because of this dual effect on physiological regulators of bone remodeling, L-arginine could potentially increase bone formation over bone resorption, and consequently, increase bone mass **(Clementi et al., 2001)**.

Glutamine has a number of unique properties suggesting that this amino acid plays an important role in health and disease. This amino acid makes more than 60% of the skeletal muscle tissue, and it is a fuel for both the digestive tract and the immune system. Also, it is playing a pivotal role in conducting nitrogen to muscle around the body **(Tapiero et al., 2002).** Glutamine may at least in part play a role in mechanisms associated with cellular proliferation and/or differentiation through particular glutamine receptors (GluR) and glutamine transporters functionally expressed in rat calvarial osteoblasts **(Yoneda and Hinoi, 2003).** The cyclization of glutamate produces proline, an amino acid important for synthesis of collagen and connective tissue **(Tapiero, et al. 2002).** Therefore, it has been suggested that glutamine may have a role in the process of bone formation.

Significant amount of taurine is transported to bone tissue, it is reasonable to propose that taurine may play an important role in bone metabolism. Interestingly, taurine has been found to inhibit experimental bone resorption and osteoclast formation and survival **(Koide et al., 1999).** It has inhibitory effects on bacteria-stimulated osteoclast formation *in vitro*. Moreover, this amino acid has stimulatory actions on alkaline phosphatase activity and collagen synthesis **(Park et al., 2001).** It may play a role in osteoblastic differentiation as well as bone matrix formation. Taurine has anti-osteopenic effect in low Ca diet-induced osteopenia in rats, thereby promoting mineralization and finally leading to its bone anabolic action **(Yasutomi et al., 2002).**

Pharmacological mixture containing amino acids and lactose accelerates and ameliorates bone fracture healing processes. This finding is linked not only to calcium metabolism but also to different biological properties which positively contribute to good healing of bone fractures **(Fini et al., 1996).**

The principal goal of the current study was to develop alternative nutritional therapeutic modalities for the treatment of primary osteoporosis in order to avoid the serious side effects of the traditional hormone replacement therapy for osteoporosis in postmenopausal women. The suggested therapeutic opportunity included the supplementation of some promising amino acids The selected amino acids include L-arginine, L-glutamine, or taurine as effective dietary supplements for management of primary osteoporosis.

Materials and Methods:

Amino acids: L-arginine, L- glutamine, taurine and lactose were purchased from Sigma Company (U.S.A). **Experimental animals:** Adult female Sprague Dawley rats (120-150g) were obtained from Animal House Colony of the National Research Centre, Cairo, Egypt. The animals were kept in wire bottomed cage at room temperature (25± 2 ºC) under a 12h dark- light cycle and acclimated to the laboratory environment for seven days before use. Animals were fed with standard laboratory diet and water *ad libitum.* The rats were ovariectomized surgically in Hormone Department, Medical Research Division at the National Research Centre. Then, after three months following surgery, the animals were divided into 6 groups as follows: The first group; was untreated (OVX) rats and served as (OVX) control. The second group; (OVX) rats which were orally administered with (1ml/rat/day) lactose 10%. The third group; (OVX) rats which were orally administered with L-arginine dissolved in 10%lactose in a dose of 500mg/kg/day **(Gupta et al., 2005).** The fourth group; (OVX) rats which were orally administered with L-glutamine dissolved in 10% lactose in a dose of 3.2g/kg/day **(Ann et al., 2004).** The fifth group; (OVX) rats which were orally administered with taurine dissolved in 10% lactose in a dose of 50mg/kg/day **(Centiner et al., 2005)**. Additional untreated gonad intact control group was involved in the present study. The experiment lasted for 3 months.

At the end of the experimental period, the animals were kept fasting for 12 hours and the blood samples were collected from the retro-orbital venous plexus under diethyl ether anesthesia **(Schermer, 1967)**. The blood samples were left to clot and the serum were separated by cooling centrifugation (4º C) at 3000 rpm for 10 min. Serum parathyroid hormone (PTH) was estimated by ELISA procedure according to the method described by **Blum et al. (1993)**. Serum 1, 25-dihydroxyvitamin D_3 (Vitamin D_3) was determined by Radio immuno assay (^{125}I RIA) according to the method of **Hollis (1986).** Serum insulin-like growth factor-1 (IGF-1), transforming growth factor-β (TGF-β) were

determined using ELISA procedure according to the method of **Blum et al. (1993) and Kim et al. (1994)** respectively. The right femur bone of each animal was dissected, cleaned and stored in formalin buffer 10% for measuring bone mineral density (BMD) and bone mineral content (BMC) using dual energy X-ray absorptiometry (DEXA). The left femur bone was also carefully removed cleaned and stored in 10% formic acid solution as a decalcifying agent for 10 days for histological investigation.

Histological Examination:

The left femur was embedded in paraffin wax and the microscopic sections of 5µm intervals were taken and stained with hematosylin and eosin (H & E) for histological examinations (**Drury and Wallington, 1980**).

Statistical Analysis:

In the present study, all results were expressed as mean ± S.E of the mean. Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version 11 followed by least significant difference (LSD) to compare significance between groups (**Armitage and Berry, 1987**). Difference was considered significant when P value ≤ 0.05.

Results:

1- Effect of Amino Acids Supplementation on PTH and Vitamin D:

Our data indicated that ovariectomy induced significant increase in serum PTH level associated with significant decrease in serum 1, 25 $(OH)_2D_3$ level in comparison with gonad intact control group. Treatment of ovariectomized rats with arginine, glutamine or taurine caused significant decrease in PTH serum level and significant increase in 1, 25 $(OH)_2D_3$ serum level comparing with ovariectomized rats that administered lactose only (Table 1).

Table (1): Effect of Different Amino Acids Supplementation on Serum Parathyroid Hormone (PTH) and 1,25 $(OH)_2D_3$ Levels in Ovariectomized Rats.

Parameters / Groups	PTH Pg/ml	1,25 $(OH)_2D_3$ (Pg/ml)
Gonad intact control	36.6±2.8	15.7±0.3
OVX Control	62.5±2.4 [a]	12.8±0.2 [a]
OVX + Lactose	60.4±2.1	13.2±0.25
OVX + Arg.	50.2±2.4 [b]	15.3±0.34 [b]
OVX + Glut.	49.4±1.7 [b]	15.2±0.18 [b]
OVX + Tau.	45.9±2.8 [b]	15.7±0.14 [b]

a : Significant change at P 0.05 in comparison with gonad intact control.
b : Significant change at P 0.05 in comparison with ovariectomized received lactose group.
OVX.: Ovariectomized rats, Arg.: Arginine, Glu.: Glutamine, Tau.: Taurine

Table (2): Effect of Different Amino Acids Supplementation on Serum Insulin-like Growth Factor-1 (IGF-1), and Transforming Growth Factor-β (TGF-β) Levels in Ovariectomized Rats.

Parameters / Groups	IGF-1 Ng/ml	TGF-β Pg/ml
Gonad intact control	9.7±0.7	193.9±9.1
OVX Control	6.7±0.32 [a]	125.8±2.7 [a]
OVX + Lactose	7.0±0.19	131.1±2.3
OVX + Arg.	8.2±0.21 [b]	160±4.0 [b]
OVX + Glut.	8.5±0.16 [b]	171.9±2.7 [b]
OVX + Tau.	8.8±0.22 [b]	179.8±2.1 [b]

a : Significant change at P 0.05 in comparison with gonad intact control.
b : Significant change at P 0.05 in comparison with ovariectomized received lactose group.
OVX.: Ovariectomized rats. Arg.: Arginine, Glu.: Glutamine, Tau.: Taurine

2- Effect of Amino Acids Supplementation on IGF-1 and TGF-β:

The present data revealed that ovariectomy resulted in significant decrease in each of IGF-1 and TGF-β in serum as compared to gonad intact control group. Treatment with the tested amino acids produced significant increase in IGF-1 and TGF-β serum levels in comparison with ovariectomized rats received lactose only as represented in Table (2).

3- Effect of Amino Acids Supplementation on BMD and BMC:

The results in table (3) showed that ovariectomy decreased BMD of proximal, mid and distal areas significantly in comparison with gonad intact control group. While ovariectomized rats treated with arginine showed significant increase in BMD of proximal and mid areas and insignificant increase in BMD of distal area in comparison with ovariectomized rats received lactose only. Treatment with glutamine or taurine induced significant increase in BMD of proximal, mid and distal areas in comparison with ovariectomized rats received lactose only. Noteworthy, ovariectomized rats treated with taurine produced significant increase in BMD of proximal, mid and distal areas in comparison with untreated ovariectomized group.

Table (3): Effect of Different Amino Acids Supplementation on Bone Mineral Density (BMD) in Ovariectomized Rats.

BMD (mg/cm^2)			
Parameters / **Groups**	Proximal mg/cm^2	Mid mg/cm^2	Distal mg/cm^2
Gonad intact control	126.4±2.5	126.2±1.9	131.0± 1.5
OVX Control	109.4±1.4 [a]	112.8±2.2 [a]	115.2± 2.9 [a]
OVX + Lactose	110.7±1.5	114.3±2.2 [a]	117.0± 2.1
OVX+ Arg.	117.2±1.9 [b]	117.6±0.7 [b]	122.6± 1.0
OVX+ Glut.	119.3±1.8 [b]	119.8±1.0 [b]	125.7± 1.0 [b]
OVX +Tau.	120.7±1.4 [b]	120.6±1.4 [b]	127.2± 1.9 [b]

a: Significant change at $P \leq 0.05$ in comparison with gonad intact control.
b : Significant change at $P \leq 0.05$ in comparison with ovariectomized received lactose group.
OVX.: Ovariectomized rats. Arg.: Arginine, Glu.: Glutamine, Tau.: Taurine

Our data in table (4) indicated that ovariectomy induced significant decrease in BMC of proximal and distal areas and insignificant decrease in BMC of mid area in comparison with gonad intact control group. Treatment with arginine, glutamine or taurine caused significant increase in BMC of proximal area comparing with ovariectomized received lactose only. While amino acids supplementation induced insignificant increase in BMC of mid area as compared to ovariectomized rats received lactose only with respect to the value of BMC of distal area only, glutamine and taurine supplementation showed significant increase, while supplementation with arginine caused insignificant increase as compared to ovariectomized rats received lactose only. It could be also seen that taurine supplementation increased BMC significantly in comparison with supplementation with arginine.

Table (4): Effect of Different Amino Acids Supplementation on Bone Mineral Content (BMC) in Ovariectomized Rats.

BMC (mg/cm^2)			
Parameters / **Groups**	Proximal	Mid	Distal
Gonad intact control	72.9±1.0	208.3±7.0	77.6±1.7
OVX. control	44.9±1.5 [a]	182±4.0	50.6±2.1 [a]
OVX + Lactose	45.4±1.0	186.8±3.2	55.8±3.3
OVX + Arg.	56.7±3.5 [b]	191.3±3.7	64.1±0.9
OVX + Glut.	59.2±3.0 [b]	195.5±2.1	67.0±0.8 [b]
OVX + Tau.	65.2±1.2 [b]	199.2±4.3	70.3±2.0 [b]

a : Significant change at $P \leq 0.05$ in comparison with gonad intact control.
b : Significant change at $P \leq 0.05$ in comparison with ovariectomized received lactose group
OVX.: Ovariectomized rats. Arg.: Arginine, Glu.: Glutamine, Tau.: Taurine

Histological Results:

Microscopic examination of left femur bone section of gonad intact control rat represented in **Fig. (1)** showed a network of bony trabiculae separated by a labyrinth of interconnecting spaces containing bone marrow. The trabiculae composed of irregular lamellae of bone with Haversian systems and lacunae containing osteocytes.

The photomicrograph of left femur bone section of untreated ovariectomized control rat in **Fig. (2)** showed the reduction of the cortical and trabicular bone thickness. Many of necrotic areas of bone and resorped cavities on the inner surface have been also seen.

Micrograph of a longitudinal section of left femur bone of ovariecomized rat received lactose **(Fig. 3)** showed some necrotic areas of bone and the presence of small cavities.

The photomicrograph of left femur section of rats treated with arginine showed the new formed bone and increase in the thickness of bone **(Fig. 4)**.

It is clear from the photomicrographs of left femur bone sections of ovariectomized rats treated with glutamine, the presence of calcified cartilage and the increased thickness of the bony trabiculae (**Fig. 5**).

A section of left femur bone of ovariectomized rat treated with taurine showed the trabiculae appeared as normal form **(Fig. 6)**.

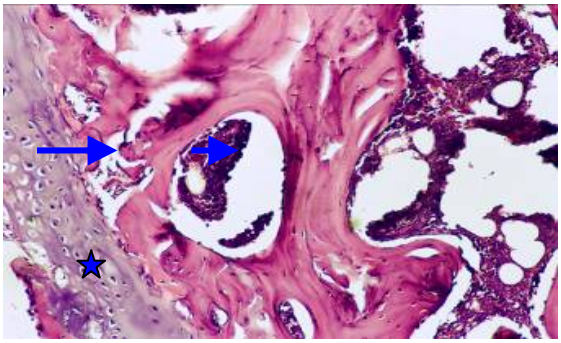

Figure (1): Photomicrograph of femur bone section of gonad intact control rat showing a network of bony trabiculae separated by a labyrinth of interconnecting spaces containing bone marrow. The trabiculae composed of irregular lamellae of bone with Haversian systems and lacunae containing osteocytes (H & E X 400).

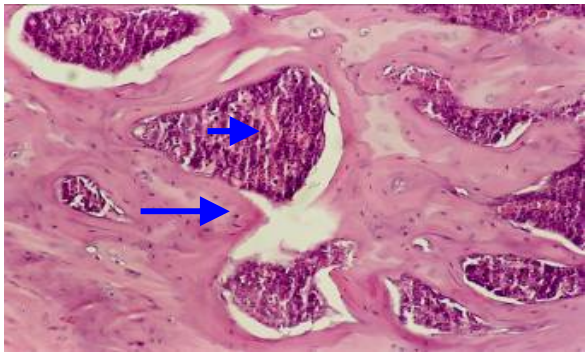

Figure (2): Micrograph of a longitudinal section of left femur bone of ovariecomized rat showing the reduction of the cortical and trabicular bone thickness. Many of necrotic areas of bone and resorped cavities on the inner surface are also seen. Cartilage layer in the trabecular bone is found (H&E x 150).

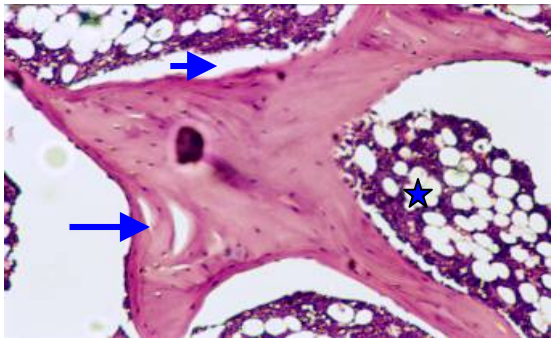

Figure (3): Micrograph of a longitudinal section of left femur bone of ovariecomized rat treated with lactose showing some necrotic areas bone and the presence of small cavities. The erosion of the outer surface of bone is also appeared (H & E X 150).

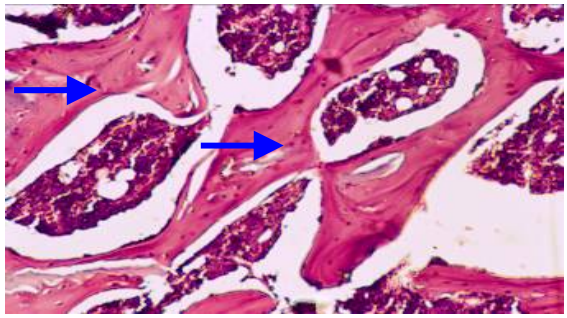

Figure (4): Micrograph of a longitudinal section of left femur bone of ovariecomized rat treated with arginine in lactose showing an increase in the thickness of bone (H & E X 150).

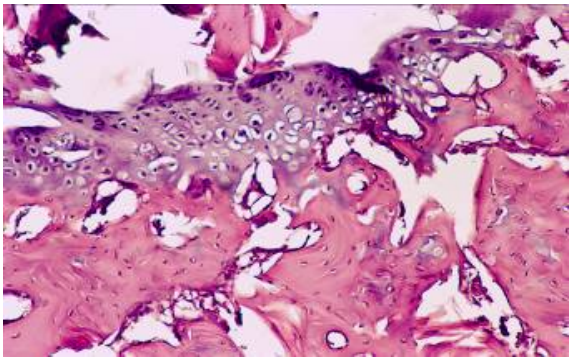

Figure (5): Micrograph of a longitudinal section of left femur bone of ovariecomized rat treated with glutamine in lactose showing the increase in the number of trabiculae (H & E X 150).

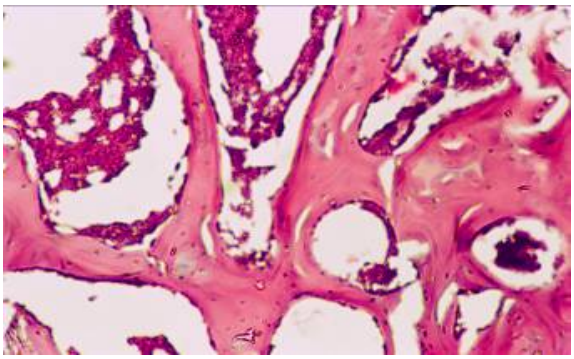

Figure (6): Micrograph of a longitudinal section of left femur bone of ovariecomized rat treated with tuarine showing normal appearance of bone (H & E X 150).

Discussion

Osteoporosis is a chronic condition chiefly affecting postmenopausal women, in whom the skeleton loses a significant percentage of its mineralized mass and mechanical resiliency, thereby becoming prone to fracture (**Fan et al., 2005).** Amino acids have been shown to stimulate bone formation and thus, they might be represented useful agents for the prevention and treatment of osteoporosis **(Conconi et al., 2001).**

The present study showed that ovarietomy induced significant decrease in all the tested parameters except PTH which increased significantly comparing with gonad intact control group. These findings are in agreement with **Segal et al, (2003) and Chen et al, (2007).** It could be explained that ovariectomy induced increase in PTH gene expression and parathyroid cell proliferation(**Silver et al., 1999).**The decrease in 1,25 $(OH)_2D_3$ serum level in ovariectomized animals may be due to that estrogen loss is the reason of reducing $1,25(OH)_2D_3$ production and calcium absorption in this rat model **(Ash and Goldin,1988),** While, the reduction of serum IGF-1 level may be explained as estrogen stimulates the autocrine secretions of IGF-1 and TGF-β by osteoblasts which are involved in the stimulation of osteoblasts maturation and growth as well as collagen synthesis and alkaline phosphatase secretion (**Kajdaniuk et al., 1999).** Many reports indicated that reduced plasma levels of IGF-1 are associated with estrogen deficiency and in turn osteoporosis in females. **Calo et al, (2000)** reported that ovariectomy resulted in significant reduction in the number of receptors for both epidermal growth factor (EGF) and IGF-1 in female rats. On the other hand, the reduction in TGF-β resulted from a direct effect of estrogen deficiency on bone cells to decrease the secretion of TGF-β with a concomitant decrease in the deposition of newly formed bone (**Finkelman et al., 1992**).. This possibility was further supported by the findings that 17β-estradiol administration directly stimulated the production of TGF-β by mouse bone cells and also estrogen replacement therapy *in vivo* corrected the TGF-β deficit (**Finkelman et al., 1992**). Moreover, it has been reported that estrogen deficiency led to a decrease in TGF-β mRNA which may simply be due to the reduction in cancellous bone volume that occurs following ovariectomy **(Westerlind et al., 1994).**

The current study also showed a reduction in BMC and BMD in ovariectomized animals as compared to the gonad intact control animals. These results may attribute to that estrogen deficiency resulted in rapid bone loss phase which is associated with loss of BMD **(Shen et al., 2000).** It has been reported that the reduced BMD in ovariectomized rats is also associated with a reduction in dry and ash femur weights as well as a decreased femoral breaking force and energy suggesting the increased risk of fracture **(Park et al., 2008).** The mechanism by which estrogen deficiency could induce bone resorption is that loss of estrogen induced enhanced expression of bone resorbing cytokines (interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α) **(Kitazawa et al., 1994)**, interleukin-6 (IL-6) **(Passeri et al., 1993)** and macrophage colony stimulating factor (M-CSF) **(Kimble et al., 1996)** by immune cells and osteoblasts in the bone marrow microenvironment **(Matsushita et al., 2008).** These cytokines are crucial for the pathogenetic mechanisms by which estrogen deficiency leads to increase the expression of functional receptor activator nuclear factor kappa B ligand (RANKL) and to enhance bone resorption and bone loss **(Kwan Tat et al., 2004),** via increasing osteoclast number and activity **(Kassam, 2003)** and promotion nuclear

factor kappa B, the key transcription factor in osteoclastogenesis **(Ross, 2003).** Ovariectomy-induced bone resorption could be responsible for the decreasing in calcium and mineral content of the whole femur **(Gaumet et al., 1996).**

Our study revealed that lactose administration in ovariectomized rats led to slight inhibition in serum PTH and slight increase in all of the other tested parameters. These results could be explained as lactose slightly increase serum level of Ca and ionized Ca which inhibit the secretion of PTH, leading to increasing the circulating level of 1,25 $(OH)_2$ D_3 by stimulating calcitriol synthesis **(Mastaglia et al., 2006).** Moreover the increase in serum IGF-1 and TGF-B levels with lactose administration may be explained as lactose has a stimulatory effect of lactose on osteoblasts growth and the production of several growth factors **(Kirk et al., 1994).**

It has been found that lactose supplementation to ovariectomised rats induced increase in BMD and BMC, which could be explained as dietary lactose increased bone calcification rate and inhibited bone resorption that lead to the improvement in skeletal growth and mineralization in animals fed lactose **(Shortt and Flynn,1991).**

Our data revealed that arginine supplementation to ovariectomized rats induced significant decrease in PTH level while, it showed significant increase in1,25$(OH)_2D_3$,IGF-1,TGF-β and both of BMD and BMC when compared with the ovariectomized rats administered lactose only. Arginine via growth hormone has been found to produce marked increase in (1, 25$(OH)_2$ D_3) due to increasing nephrogonous cyclic AMP(N_cAMP) **(Ahmad et al., 2003)**. The increase in IGF-1 level may be due to the role of arginine in stimulating IGF-1 production and collagen synthesis in osteoblasts-like cells. It has been suggested that arginine could increase IGF-1 mRNA transcription and alpha (1) collagen mRNA transcripts and thus, arginine may influence bone formation by enhancing IGF-1 production **(Chevalley et al.,1998**). Lactose appeared to have an intangable role in enhancing serum level of IGF-1 in ovariectomized rats via stimulation of osteoblasts cells growth, but the major role in this respect could be attributed to arginine supplementation. Also arginine directly increased the expression of TGF-β mRNA and TGF-β protein levels as it could increase the production and deposition of matrix components **(Narita et al., 1995).** Furthermore the increase in BMD due to arginine supplementation could be explained as arginine has a dual effect on physiological regulators of bone remodeling. Arginine could potentially increase bone formation over bone resorption, and consequently, increase bone mass **(Van't Hof and Ralston, 2001).** Additionally, there is growing evidence demonstrated that moderate concentrations of nitric oxide (NO) play an essential physiological role in promoting maintenance of bone density-stimulating new bone formation while suppressing bone catabolism **(Armour et al., 2001).**

Our data indicated that glutamine administration to ovariectomized rats tends to significantly increase 1,25$(OH)_2D_3$,IGF-1,TGF-β,BMC and BMD. While it could significantly decrease serum PTH level when compared to ovareiectomized rats administered lactose only.

It has been reported that glutamine supplementation produces glutathione which acts as a potent enhancer of calcium through activation of calcium sensing receptor (CaSR). Glutathione acts as an endogenous modulator of this receptor particularly in the parathyroid gland where this receptor is known to control parathyroid hormone release **(Wang et al., 2006).** Also glutathione could increase circulating 1,25 $(OH)_2D_3$ via stimulating enzymatic activity of the renal 1 alpha-hydroxylase of 25-hydroxycholecalciferol **(Schedl et al.,1992).** Furthermore glutamine supplementation increases IGF-1 level due to its conversion to alpha-ketoglutarate (αKG) in the body. Alpha - ketoglutarate has been shown to increase circulating plasma levels of insulin, growth hormone with consequent increase in IGF-1 **(Jeevanandam and Petersen, 1999)**. Growth hormone could stimulate osteoblastic proliferation and differentiation and increase the production of IGF-1 **(Corpas et al., 1993).**

The role of glutamine in inducing the detectable increase in BMD of right femur areas of ovariectomized rats mainly depends on its anabolic effect on bone, since glutamine played specific role in mechanisms associated with cellular proliferation and/or differentiation through particular receptors and transporters functionally expressed in rat calverial osteoblasts **(Yoneda and Hinoi, 2003).** Moreover, Alpha ketoglutrate has been found to increase mineralization, higher volumetric cortical bone density and increase trabecular bone density in animals **(Tatara et al., 2005).** Also alpha ketoglutrate is a component of antioxidant glutathione and polyglutamated folic acid. These antioxidants play a role in inhibiting osteoclastogensis via inhibition of reactive oxygen species (ROS) which are necessary for osteoclast activity and bone resorption **(Key et al., 1994)**. The positive effect of α-KG on bone was previously reported in birds since it could increase bone weight, mean relative wall thickness, maximum elastic strength, ultimate strength and volumetric bone

mineral density in birds **(Tatara et al., 2005)** Similar findings have been also reported in ovariectomized rats **(Radzki et al., 2002).** Finally, the cyclization of glutamine produces proline, an amino acid important for synthesis of collagen and connective tissue **(Tapiero et al., 2002)** which contributes to the positive influence of glutamine on bone tissue.

The suggested mode of action of α-KG on bone mineralization could be attributed to the efficacy of α-KG to maintain a delicate balance between bone resorption and bone formation that plays an important role in determining bone strength and integrity **(Rodan and Mertin, 2000).** Glutamine may play a role as a signal mediator in mechanisms associated with chondral mineralization through the group III m glutamine receptor (mGluR) subtype functionally expressed by chondrocytes in cartilage **(Wang et al., 2006).** Thus, glutamine could produce the increase in BMC of each of proximal, mid and distal areas of ovariectomized rat right femur bone through its indirect effect on mechanical properties of bone. Lactose has a significant role in promoting the effect of glutamine on BMC of the three regions of right femur bone of OVX rats in the present study.

The current results showed that taurine administration induced significant decrease in serum PTH level while it produced significant increase in each of $1,25(OH)_2D_3$, IGF-1,TGF-β,BMC and BMD in ovariecomized rats. The effect of taurine in decreasing PTH level could be attributed to its role in increasing magnesium concentration through the activation of extracellular signal regulated protein kinase (ERK) pathway **(Jeon et al., 2007).** The resulting elevation in magnesium concentration could suppress PTH secretion as magnesium positively affected intestinal calcium absorption and bone metabolism in ovariectomized rats **(Toba et al., 2000).** Taurine has been shown to have direct effect on accelerating vitamin D absorption and in turn increasing serum $1, 25(OH)_2 D_3$ level in ovariectomized rats treated with taurine. This suggestion is greatly supported by **Petrosian and Haroutounian, (2000). Gaylord et al. (2007)** stated that taurine stimulated pituitary growth hormone with subsequent stimulation of growth hormone – dependent IGF-1 in animals. Thus, growth hormone responsive and IGF-1 secreting cells might require sufficient taurine to secrete IGF-1 at normal levels **(Hu et al., 2000).** Taurine could produce the detectable increase in serum TGF-β level by two mechanisms, stimulatory action of taurine on osteoblastic differentiatation as well as bone matrix formation **(Park et al., 2001),** and indirect effect of taurine in increasing circulating level of $1,25 (OH)_2 D_3$ which in turn led to increasing TGF-β release from bone cells. Lactose may have a role in enhancing TGF-β level in contribution with taurine through stimulation of osteoblast growth and production of growth factors mainly TGF-β **(Petrosian and Haroutounian, 2000).** Moreover taurine has been found to promote osteoblasts mineralization and it could regulate osteoblasts metabolism via stimulation of extracellular signal regulated protein kinase phosphorylation **(Park et al., 2001).** Taurine might also have antiresorptive action through its antioxidant effect **(Lourenco and Camilo, 2002).** Therefore, we could suggest that taurine via scavenging reactive oxygen species, necessary for osteoclast function, could inhibit bone resorption. Therefore, through these common pathways, taurine has a preventive effect on bone loss. The unique role of taurine in modulating mitochondrial Ca^{2+} homeostasis might be of particular importance under pathological conditions **(Palmi et al., 1999).** Lactose may have a synergistic effect with taurine on increasing BMC of proximal, mid and distal regions of right femur bone.

Histological investigation of bone tissue sections showed that estrogen deficiency is associated with elevated bone resorption caused by a rise of osteoclast number**.** Recent study of **Park et al. (2008)** observed that the ovariectomized rats that exhibited osteoporosis within 7 weeks after surgery showed large decreases in the bone volume ratio and trabecular bone thickness.

Treatment with lactose showed some necrotic areas and appearance of small cavities in the bone. The ability of lactose to facilitate the passage of calcium across the intestine, resulting in improved calcium availability to the skeleton **(Miller et al., 1988). Marie and Travers (1983)** observed a slight decrease in osteocytes thickness in rats fed diet containing lactose.

Supplementation of ovariectomized rats with arginine revealed the formation of new bone. This finding could be explained in the view of the effect of arginine via growth hormone stimulation as well as IGF-1 production. Growth hormone has been found to have a positive effect on chondrocytes and osteoblasts **(Saggese et al., 1995)** as well as it could increase the number and function of osteoblasts **(Bouillon, 1991).** This is because of osteoblasts express functional growth hormone receptors (GHR) **(Nilsson, et al., 1995)** indicating that growth hormone (GH) also exerts a direct effect on osteoblasts. A direct effect of GH on osteoblasts is supported by earlier results for the epiphysial growth plate, where it has been demonstrated that GH interacts directly with epiphysial chondrocytes for the regulation of longitudinal bone growth **(Ohlsson et al., 1992)**.IGF-1 has been found to play a role in trabecular and cortical bone formation **(Conconi et**

al., 2001) IGF-1 showed a positive effect on bone formation in vitro as it could stimulate the formation of osteocalcin, collagen and non-collagenous matrix proteins by differentiated osteoblasts and increased the number of functional osteoblasts by promoting osteoprogenitor cell replication **(Visser and Hoekman 1994)**. Lactose assisted arginine in mainting plenty of calcium for strengthening the fragile bone, and increasing bone thickness as appeared in the current study.

Supplementation of ovariectomized rats with glutamine showed the calcification of cartilage, increased trabecular bone thickness and formation of new bone. This result could be attributed to the functional role of glutamine in chondral mineralization through the group III mGluR subtype functionally expressed by chondrocytes in cartilage **(Wang et al., 2006).** Moreover, glutamine via producing α-KG in the body could promoting bone weight, wall thickness and bone strength **(Tatara et al., 2005).** Recent study of **Polat et al. (2007)** demonstrated that glutamine had positive effects on healing of traumatically fractured bone through attainment of positive nitrogen balance. The role of lactose here is to improve calcium availability to facilitate the formation of new bone.

Taurine could restore the normal appearance of trabiculae as shown in the micrograph of bone tissue section of left femur of ovariectomized rats treated with taurine in the current work. Considering that a significant amount of taurine is transported to bone tissues, the transcription and translation of taurine occurs in bone forming cells **(Yuan et al., 2006)**. Therefore, it is reasonable to propose that taurine may play an important role in bone metabolism. Taurine in the osteoblasts activates extracellular signal regulated protein kinase-2 (ERK2) and phosphorylates transcription factors thus activating collagen gene transcription and protein synthesis. These actions of taurine may be beneficial for osteoblastic differentiation and bone matrix formation (**Park et al., 2001**). Additionally, taurine could stabilize cell membranes, eliminate oxide free radicals, regulate intracellular osmosis and maintain intracellular calcium concentration (**Pasantes-Morales et al., 1998**). It is clear that lactose could enhance the effect of taurine on bone formation through improving the organization of the trabecular bone in ovariectomized rats as shown in the present study from the normal appearance of bone following administration of taurine in lactose.

In conclusion, the present study provided clear evidence that ovariectomy produced marked abnormalities in bone biomarkers and in increasing risk of fracture. Also ovariectomy reduced plasma IGF-1 level and decreased TGF-β, as well as accelerated bone loss phase. The selected amino acids showed positive effect on bone via inhibiting the secretion of parathyroid hormone in concomitant with increasing serum 1,25 dihydroxyvitamin D_3 Also, the studied amino acids stimulated the production of IGF-1 and TGF-β and increased bone calcification rate as well as improved calcium availability to the skeleton. Lactose participated in enhancing the positive effect of the selected amino acids on bone. Arginine provided promising effect on bone through stimulation of insulin- like growth factor. Also, arginine via nitric oxide which is involved in increasing basal calcium absorption in small intestine thus stimulates the replication of primary osteoblasts and as well as inhibiting osteoclasting bone resorption. Glutamine through the production of alpha-ketoglutarate and glutathione showed potent effect on bone. These metabolic products of glutamine have a critical role in increasing bone density and strength in addition to bone mineralization. Taurine revealed the most effective action on bone remodeling via stimulating osteoblastic differentiation as well as bone matrix formation. In addition to taurine anabolic effect, it has an antiresorptive action through its antioxidant activity which participates in inhibiting osteoclast function and consequently bone resorption. Our finding might be useful for the future strategies against menopausal bone turnover and implicitly osteoporosis progression.

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