Research Paper Physiology

Comparison of L-Carnitine Supplementation and α-Tocopherol in Restoring Bone in Rat Model of Osteoporosis

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ABSTRACT

Previous researches have reported that both of carnitine and vitamin E have significant effects on bone mass, volume, and microarchitecture. This study compares between the individual effects of L-carnitine and tocopherol on bone mass and structure in the ovariectomized (OVX) rat model of osteoporosis and also, evaluates their combined effects. Once bone loss had been established, rats were divided into five groups: Sham, OVX, and 3 treated groups using L-carnitine or α-tocopherol or both. At the end of the study period, rats were killed and blood samples were collected for biochemical analysis of serum calcium, alkaline phosphatase, osteocalcin (bone remodeling markers), and Cross- linked C-terminal telopeptide (bone resorption marker). Both tibias and the 4rd and 5th lumbar vertebrae were excised for dual energy X-ray absorptiometry (DEXA) and histological examination. The result of this study demonstrated that L-carnitine was able to restore the bone loss in ovariectomized rat model of osteoporosis while, α-tocopherol was unable to reverse the loss of bone in the same animal model. Moreover, combined treatment with L-carnitine and α-tocopherol showed more improvement in bone mass and bone remodeling markers compared to L-carnitine or α-tocopherol alone. Therefore, combination of L-carnitine and α-tocopherol has potential for the treatment of post-menopausal osteoporosis.

KEYWORDS : Osteoporosis, L-carnitine, α-tocopherol, bone mineral density, cortical bone thickness, trabecular bone density

Introduction:

Bone is a highly dynamic tissue that is continuously remodeling to respond to altered demand for structural support and also to maintain blood calcium homeostasis. Normally there is a balance between the processes of bone formation and resorption so; osteoporosis takes place when bone resorption by osteoclasts far exceeds bone formation by osteoblasts resulting in bone fragility (low bon mass and micro architectural deterioration of bone tissue) with increasing susceptibility to fracture (1). It is known that osteoporosis can be caused by various endocrine, metabolic, and mechanical factors. Post-menopausal bone loss remains the most common cause of osteoporosis During the first 5–10 years after menopause, a physiological state exists in which bone turnover (i.e., both resorption and formation rate) is accelerated with the catabolic activity of osteoclasts favored over the anabolic activity of osteoblasts. So, women were found to have a higher risk of getting osteoporosis than men with the ratio of 1.6:1 (2).

At present, the approach to osteoporosis management is aimed at preventing fractures from taking place and stabilizing bone metabolism. Nonetheless, not a single agent is able to maintain bone mass and density without exerting undesirable side effects. Current available therapies are effective in the prevention of bone loss by stabilizing the bone mass through inhibition of osteoclast activity, but they are not favored to treat established osteoporosis where there is a need to increase bone volume. However, intermittent parathyroid hormone is considered an anabolic agent as it acts to increase bone formation and mineralization by upregulating osteoblast differentiation and activity (3). However, the use of parathyroid hormone is associated with some drawbacks such as daily injection and the possibility of tumorigenesis (4). Although Estrogen replacement therapy is considered the main form of treatment and prevention of post- menopausal osteoporosis but it has some restrictions due to the potential risk of breast cancer, venous thromboembolism, migraine, coronary heart disease and strokes (5). Besides these, its effect on bone mineral density (BMD) depends greatly on the time when the therapy is initiated, and the total duration of its use (6). Because of increasing incidence of osteoporosis, especially in Western countries, and the need for better treatment options, the search continues for alternative strategies to maintain and restore skeletal health.

Antioxidant vitamins can potentially be used to treat and prevent the progress of osteoporosis. Vitamin E, a fat-soluble antioxidant, has been of particular interest due to its ability to suppress the production of certain proinflammatory mediators that have been linked to increased bone loss, and protect against oxidative damage (7). The relationship between vitamin E and bone has been studied to some extent. Feresin et al. (8) reported that vitamin E supplementation is able to prevent bone loss and improve bone biochemical properties in ovariectomized (OVX) rats. Also, Mohammad et al. (9) demonstrated that vitamin E either in the form of tocopherol or tocotrienol prevents bone loss and inhibits osteoclastogenesis in OVX rats by suppressing receptor activator of nuclear factor κB (RANKL) and inhibiting the release of bone-resorbing cytokines. Furthermore, the administration of tocopherol was shown to suppress the production of oxygen derived free radicals (ODFRs) in the early stages of fracture healing in rabbits (10), increase the activity of antioxidant enzymes in Ovx rats (11), and enhance bone fracture healing in its early and late phases in Ovx rats and in male rats (12). Another cross-sectional study reported that vitamin E causing an increase in the rate of bone formation while having no effects on the rate of bone resorption in post-menopausal women (13). In contrast, a study done by Fujita et al. found that tocopherol increases bone breakdown and reduces bone mass through stimulating the formation of osteoclasts (14).

Carnitine is an essential cofactor for the transport of long-chain fatty acids across the inner mitochondrial membrane into the mitochondrial matrix for β-oxidation, which is the most efficient metabolic pathway for energy production (15).Osteoblast activity is depend on energy production. Thus, the loss of energy production which accompanies age may account for decreased osteoblast activity. Some researchers have studied the effects of carnitine on bone. Hooshmand et al. (16) reported that dietary l-carnitine supplementation improves bone mineral density by suppressing bone turnover in aged ovariectomized rats. Also, administration of carnitine was shown to reduce bone loss and improved inflammatory biomarkers in ovariectomy and inflammation induced osteoporosis in rats(17), accelerate the recovery of bone volume/total volume ratio in osteoporotic pregnant mice (18), and positively affect human osteoblast proliferation and differentiation in vitro (19).

The purpose of this study were to compare between the individual effects of L-carnitine and tocopherol on bone mass and structure in the OVX rat model of osteoporosis and also to evaluate their combined effects.

Material and methods

Forty-six, virgin Sprague Dawley female rats with an average weight 180–200 mg were utilized for this study. Rats were obtained from the Experimental Animal House of Mansoura University. Virgin rats were chosen to limit variability in skeletal status compared to already active breeders caused by multiple earlier breeding. Animals were housed in standard cages, three per cage, and maintained under controlled conditions of light (12 h dark–light cycles) and temperature

(25°C). Following a 7-day acclimation period, rats were anesthetized using a ketamine/xylazine cocktail (80 mg ketamine and 8 mg xylazine/kg body weight) for either a sham operation (Sham), for simulation of surgical stress, or bilateral ovariectomy (OVX). Ovariectomy was performed according to the method described in Waynforth (20). A subset of Sham and OVX animals (n = 6/ group) were sacrificed after 2 weeks to confirm bone loss by assessment of tibia and spine BMD. Once bone loss had been established, rats were divided into the following groups (n = 8 rats/group): Sham group, OVX group, OVX rats treated with L-carnitine (OVX+C) at a dose of 50 mg/kg, OVX rats treated with vitamin E (α-tocopherol acetate) at a dose of 60 mg/kg dissolved in 1 ml vegetable oil (OVX+AT) and OVX rats treated with both carnitine and α-tocopherol (OVX+C+AT) in the same previous doses. Both α-tocopherol and -L-carnitine were given orally to the rats using an oral gavage needle once daily for 8 weeks.

At the end of the study period, rats were killed by cervical dislocation. Blood samples were obtained directly from the heart. Blood immediately centrifuged and serum will collected and stored at -20°c until biochemical analysis. Both tibias and the 4rd and 5th lumbar vertebrae were excised and cleaned of soft tissue. Bone densitometry was performed (tibia and vertebra). Tibias from the left hindlimb and 4th lumbar vertebra were analyzed for structural alterations by histopathological examination. Body weights were measured for all rats all over the period of the study.

The experimental protocol was approved by the Institutional Animal Ethics Committee of Mansoura University and care of the animals was carried out as per the guidelines of the Committee. All sacrificed animals were disposed in a safety cabinet in the Medical Experimental Research Centre in Mansoura University.

Both α-tocopherol and L-carnitine were purchased from Sigma Chemical Company (USA)

Bone Densitometry

Right tibia from each rat and 5th lumber vertebra were stored in formalin buffer 10% for dual energy X-ray absorptiometry (DEXA). Bone mineral density (BMD), area (BMA), and content (BMC) were measured by dual - energy X - ray absorptiometry (DEXA) using Norland XR-46, version 3.9.6/2.3.1 instrument (Norland X-R-46 version 3.9.6, Peachtree City, GA, USA) equipped with dedicated software for small animal measurements.

Serum Measurements

Serum calcium was measured by an Olympus AU 600 model autoanalyzer. While serum alkaline phosphatase (ALP) concentration was measured by colorimetric method (Chemroy, Biochemical Trade, Inc. USA) in Beckman Coulter DU-70 spectrophotometer (Beckman Coulter Inc., CA, USA). Serum Osteocalcin concentration was measured by Rat Osteocalcin ELISA Kit (Biochemical Technologies, Inc., Stoughton, MA, USA) and according to manufacturer's instructions. The sensitivity of osteocalcin measurement is 0.5ng/ml and the intra- and inter-assay variability were 4% and7% respectively. Serum Cross- linked C-terminal telopeptide (CTX) was measured by Rat CTX ELISA Kit (MyBioSource, Inc., San Diego, California, USA) and according to manufacturer's instructions. The sensitivity of CTX measurement is 12pg/ml and the intra- and inter-assay variability were <= 8% and <= 12% respectively.

Histology of the Bone

Tibias and 4th lumbar vertebrae were fixed in 10% formalin for 48 hours and then transferred to 70% ethanol. Tibias and vertebrae were then decalcified in Cal-Rite and processed by dehydration through sequential ethanol steps, cleared with toluene, and paraffin embedded. Sections of 5µm thickness were prepared and stained with haematoxylin and eosin (H&E) for image analysis and to examine morphology. All slides were examined under light microscopy at low (X40) and high (X100) magnification.

Image analysis procedure* (*computer Assisted digital image analysis)

Slides were photographed using Olympus® digital camera installed on Olympus® microscope with 1/2 X photo adaptor, using 20 X objective. The result images were analyzed on Intel® Core I3® based computer using VideoTest Morphology® software (Russia) with a specific built-in routine for area and calibrated distance measurement. 5 slides from each case were prepared, 5 random fields from each slides were analyzed. All images were calibrated against a micrometer slide photographed with the same system under the same magnification. Trabecular bone was manually selected and extracted from empty areas using Genius® G-Pen F509® digital tablet then trabecular bone density (TBD)(%) and trabecular bone area (TBA) (% against total bone in the field) were calculated. Cortical bone thickness (CBT) was measured using manual line measuring tool which is calibrated against a micrometer slide photographed under the same conditions and expressed in µm.

Statistical analysis

Data were collected from repeated experiments and are presented as mean ± SD. A one-way analysis of variance (ANOVA) followed by Tukey's post hoc test were used for statistical analysis. Analysis was performed using the SPSS software (ver. 17.0, IBM, Chicago,IL, USA). P values ≤0.05 were considered statistically significant.

Results:

Table1. Body weight (g) in different groups

n=8	Sham	OVX	OVX+AT	OVX+C	OVX+C+AT	ANOVA P value
Mean	217.7	273.6*	269.3*	212.8#	214.4#	<0.0001
SD	±11.1	±15.1	±18.0	±11.1	±12.8	

Values are expressed as mean ±SD. Non-significant: at P>0.05. Significant: at P<0.05. * = significance between sham group and other groups; # = significance between OVX group and treatment groups.

Dual x-ray absorptiometric analyses revealed that rats in the OVX group had lower bone mineral density and bone mineral content than those in the sham operated group both in the 5th lumber vertebra and tibial bone (Tables 3 and 4). Also, in Tables 2 OVX group showed significant increase in alkaline phosphatase, osteocalcin, and CTX serum levels (P≤0.05) with insignificant change in serum calcium level. Image analysis examination of bone tissue from ovariectomized rats confirmed trabecular bone loss (tables 5, 6) in association with enlarged fat cell accumulation, multiple osteopenic bony bits in the bone marrow compartment and less osteoblastic activity (Figure 2).

Table2. Effect of L-carnitine and α-tocopherol on serum measurements in rat model of osteoporosis.

n=8	Sham	OVX	OVX+AT	OVX+C	OVX+C+AT	ANOVA P value
Calcium (mg/dl)	9.83±1	9.20±51	9.45±53	9.46±35	9.24±24	0.45
ALP (U/L)	40.2±4.5	49.8±4.9	59.7±6.6*#	67.4±6.3*#	69.0±6.6*#$	<0.0001
Osteocalcin (ng/ml)	16.5±2.4	23.2±4.5*	26.7±1.4*	32.2±4.7*#$	43.3±2.1*#$∞	<0.0001
CTX (ng/ml)	27.5±1	63.6±1.8*	51.6±3.5*#	37.3±1.4*#$	29.2±0.9#$∞	<0.0001

Values are expressed as mean ±SD. Non-significant: at P>0.05. Significant: at P<0.05. Alkaline phosphatase (ALP) and cross- linked c-terminal telopeptide (CTX). * = significance between sham group and other groups; # = significance between OVX group and treatment

groups; $ = significance between OVX+AT group and OVX+C group or OVX+C+AT group; ∞ = significance difference between OVX+C group and OVX+C+AT group.

Treatment with α-tocopherol cannot improve the deterioration in BMD, BMC, CBT, mean TBD, and trabecular area versus total bone area in the field resulted from ovariectomy (Tables 3, 4, 5, 6 and figure 3). However, OVX+AT group showed significant decrease in serum CTX and significant increase in serum ALP with insignificant change in osteocalcin levels as compared to OVX group.

On the contrary, the other treatment groups (OVX+C and OVX+C+AT) showed significant improvement in BMD, BMC, CBT, TBD, and trabecular area versus total bone area ratio (P≤0.05) as compared to OVX group. They also, showed significant elevation in serum ALP, osteocalcin levels and reduction in serum CTX levels (P≤0.05).

Table3. Effects of L- carnitine and α-tocopherol on DEXA bone parameters of fifth lumbar vertebrae in ovariectomized rats.

n=8	Sham	OVX	OVX+AT	OVX+C	OVX+C+AT	ANOVA P value
BMD (g/cm^2)	0.378±0.02	0.217±0.03*	0.229±0.02*	0.331±0.04#$	0.384±0.01#$∞	<0.001
BMC (g)	0.186±0.01	11.2±0.02*	0.132±0.01*	0.165±0.02*#$	0.191±0.02#$∞	<0.001
BMA (cm^2)	0.760±0.07	0.662±0.09*	0.680±0.08*	0.717±0.09#	0.738±0.09#	0.01

Values are expressed as mean ±SD. Non-significant: at P>0.05. Significant: at P<0.05. Bone mineral density (BMD), bone mineral content (BMC), and bone mineral area (BMA). * = significance between sham group and other groups; # = significance between OVX group and treatment groups; $ = significance between OVX+AT group and OVX+C group or OVX+C+AT group; ∞ = significance difference between OVX+C group and OVX+C+AT group.

Table4. Effects of L- carnitine and α-tocopherol on DEXA bone parameters of tibial bone in ovariectomized rats.

n=8	Sham	OVX	OVX+AT	OVX+C	OVX+C+AT	ANOVA P value
BMD (g/cm^2)	0.175±0.01	0.121±0.01*	0.123±0.01*	0.162±0.01#$	0.166±0.02#$	<0.001
BMC (g)	0.273±0.02	0.206±0.02*	0.216±0.03*	0.230±0.05*#	0.241±0.02#$	<0.001
BMA (cm^2)	0.229±0.05	0.233±0.08	0.239±0.09	0.231±0.07	0.250±0.08	0.99

Values are expressed as mean ±SD. Non-significant: at P>0.05. Significant: at P<0.05. Bone mineral density(BMD), bone mineral content (BMC), and bone mineral area (BMA). * = significance between sham group and other groups; # = significance between OVX group and treatment groups; $ = significance between OVX+AT group and OVX+C group or OVX+C+AT group.

Moreover, the present results revealed that the group of rats receiving combined treatment with L- carnitine and α-tocopherol showed higher bone mass and more improvement in bone remodeling markers (ALP, osteocalcin, and CTX) than those receiving L-carnitine alone.

As regarded to serum calcium levels all groups of the study showed insignificant change in its level.

All groups of rats showed a consistent increase in body weight throughout the study period. However, the OVX and OVX+AT groups showed a significant increase in body weight (P≤0.05) at the end of the study compared to the other groups (table 1).

Table5. Effects of L- carnitine and α-tocopherol on CBT, TBD, and TBA of 5th lumbar vertebra in ovariectomized rats.

n=8	Sham	OVX	OVX+AT	OVX+C	OVX+C+AT	ANOVA P value
CBT (μm)	295.9±18.1	185.8±18.1*	188.7±17.7*	250.6±14.4#$	267.7±23.5#$	<0.001
TBD (%)	85.53±13.7	37.98±9.4*	42.99±11.1*	48.87±7.6*	72.63±6.1#$	<0.001
TBA (%)	63.17±9.4	18.69±9.7*	20.13±5.0*	39.54±9.2*	55.94±9.3#$	<0.001

Values are expressed as mean ±SD. Non-significant: at P>0.05. Significant: at P<0.05. Cortical bone thickness (CBT), trabecular bone density (TBD), and trabecular bone area versus total bone area in the field (TBA). * = significance between sham group and other groups; # = significance between OVX group and treatment groups; $ = significance between OVX+AT group and OVX+C group or OVX+C+AT group.

Table6. Effects of L- carnitine and α-tocopherol or both on CBT, TBD, and TBA of tibial bone in ovariectomized rats.

n=8	Sham	OVX	OVX+AT	OVX+C	OVX+C+AT	ANOVA P value
CBT (μm)	251.5±16.2	155.2±23*	175.8±12.4*	197.3±11.2#	223.1±20.7#	<0.001
TBD (%)	75.18±6.0	32.14±3.4*	40.60±5.6*	45.74±5.2*#	62.73±7.0#$∞	<0.001
TBA (%)	65.64±4.3	23.94±3.9*	35.53±4.3*	39.52±5.2*#	56.79±4.5#$∞	<0.001

Values are expressed as mean ±SD. Non-significant: at P>0.05. Significant: at P<0.05. Cortical bone thickness (CBT), trabecular bone density (TBD), and trabecular bone area versus total bone area in the field (TBA). * = significance between sham group and other groups; # = significance between OVX group and treatment groups; $ = significance between OVX+AT group and OVX+C group or OVX+C+AT group; ∞ = significance difference between OVX+C group and OVX+C+AT group.

A) Vertebra B) Tibia

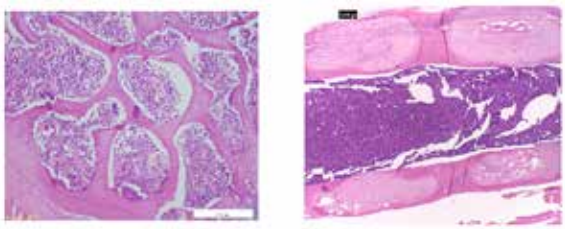

Figure1. The photomicrograph of a section in the vertebra (A) and tibia (B) of sham group showing mean cortical bone thickness, mean trabecular bone density and mean trabecular bone area versus total bone area. Hx. & E.; ×100.

A) Vertebra B) Tibia

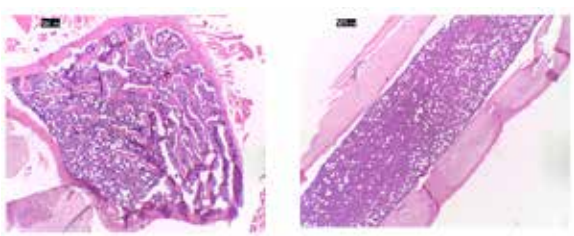

Figure2. The photomicrograph of a section in the vertebra (A) and tibia (B) of ovariectomized rats showing a significant decrease in mean cortical bone thickness, mean trabecular bone density and mean trabecular bone area versus total bone area in association with enlarged fat cell accumulation in the bone marrow compartment. Hx. & E.; ×100.

A) Vertebra B) Tibia

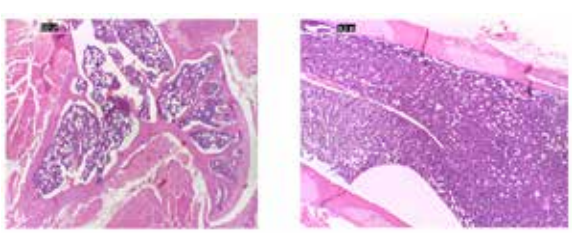

Figure3. The photomicrograph of a section in the vertebra (A) and tibia (B) of rats receiving α-tocopherol showing a significant bone loss in association with enlarged fat cell accumulation in the bone marrow compartment. Hx. & E.; ×100.

A) Vertebra B) Tibia

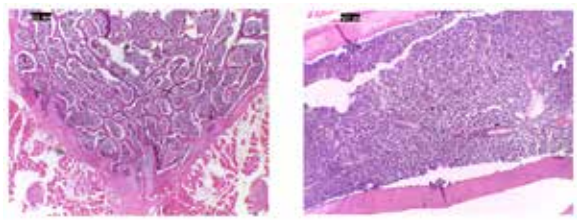

Figure4. The photomicrograph of a section in the vertebra (A) and tibia (B) of rats receiving L- carnitine showing a significant improvement in mean cortical bone thickness, mean trabecular bone density and mean trabecular bone area versus total bone area. Hx. & E.; ×100.

A) Vertebra B) Tibia

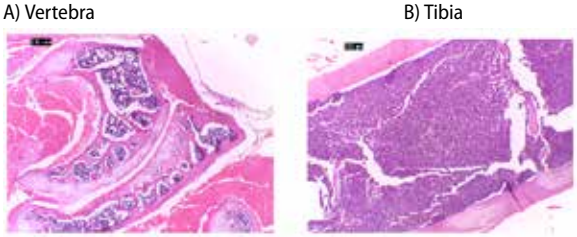

Figure5. The photomicrograph of a section in the vertebra (A) and tibia (B) of rats receiving L- carnitine plus α-tocopherol showing a significant improvement in mean cortical bone thickness, mean trabecular bone density and mean trabecular bone area versus total bone area. Hx. & E.; ×100.

Discussion:

Osteoporosis is known as a silent age-related disorder, and it is considered as a major public health problem. Most of the current available therapies have undesirable side effects and are not able to treat established osteoporosis. Previous researches have reported that both of carnitine and vitamn E have significant effects on bone mass, volume, and microarchitecture. This study compares between the individual effects of L-carnitine and α-tocopherol on bone mass and structure in the OVX rat model of osteoporosis and also, evaluates their combined effects. The ovariectomized rat was used in this study as a model of post-menopausal osteoporosis because its bone loss resembles the biphasic bone loss in human, with initial rapid phase of bone loss occurring up to 100 days. Moreover, OVX rats can serve as an animal model to investigate the effects of therapeutic agents on bone mass, structure and turnover (21).

In this study, loss of bone with an increase in resorption and formation markers was seen in ovariectomized rats. The DEXA and morphometry bone results showed that bilateral ovariectomy was associated with low BMD, BMC, CBT, TBD, and mean TBA compared to the sham group with suggestion of low bone turnover and the high susceptibility of fractures. These findings in post-menopausal rat model are consistent with previous studies (22, 23).

The results of the current study showed that daily supplementation of with α-tocopherol was not able to restore the loss of BMD and BMC or reverse the decrease in CBT, TBD, and mean TBA in the 4th lumber vertebra and tibial bone due to ovariectomy. These findings agreed with other researches such as Chai et al. (24) and Feresin et al.(8) who found that vitamin E could not alter the decrease in bone volume/

total volume ratio(BV/TV), trabecular number(Tb.N), trabecular thickness (Tb.Th) and the increase in trabecular separation (Tb.Sp) in orchidectomized male rats and ovariectomized female rats respectively. Contrary to our findings, few studies have reported that vitamin E positively influence bone quality. For instance, Hermizi et al. (25) have demonstrated that supplementation with vitamin E lowered the bone resorption parameters including osteoclast/bone surface (Oc.S/BS) and eroded surface/bone surface (ES/BS) in male rats after receiving nicotine in comparison to control group. Also, Ahmad et al. (26) reported that vitamin E is able to decrease ES/BS in male rats receiving ferric nitriloacetate. Additionally, Shuid et al. (27) have shown that supplementation with vitamin E significantly increases BV/TV, Tb.Th, and Tb.N and decreases Tb.Sp in normal male rats. However, it is important to mentioning that all the previous studies which demonstrated beneficial effects of vitamin E on bone were examining the role of vitamin E in preventing bone loss but not its reversal.

On the other hand, treatment with L-carnitine showed significant increase in, BMD, BMC, CBT, TBD and mean TBA both in vertebral and tibial bones as compared to OVX group. These results are consistent with Patano et al. (18) who reported that L- carnitine and isovaleryl-L-carnitine accelerated the recovery of bone volume/total volume ratio after experimentally induced osteoporosis in pregnant mice. Also, Hooshmand et al. (16) found that dietary L-carnitine supplementation improved bone mineral density by suppressing bone turnover in aged ovariectomized rats. Moreover, administration of carnitine was shown to positively affect human osteoblast proliferation and differentiation in vitro and protect against apoptosis of murine MC3T3-E1 osteoblastic cells (28).

Carnitine facilitates energy availability, and it is particularly important for those tissues with high energy requirements. Adamek et al. (29) demonstrated that cells of the osteoblastic lineage generate 40-80% of the energy demands through fatty acid oxidation. Thus, modulation of fatty acid oxidation will regulate osteoblast activity and the amount of energy available for protein synthesis in osteoblasts. Furthermore, L-carnitine (LC) could modulate proliferation and differentiation of osteoblasts in vitro through the involvement of insulin-like growth factors (IGFs) and IGF binding proteins (IGFBPs). Colucci et al. (19) have reported that carnitine modulated IGFBPs by increasing the release of IGFBP-3 and decreasing the release of IGFBP-5, which exert, respectively, a stimulatory and an inhibitory effect on osteoblast proliferation and activity. Moreover, possible additional mechanisms including the antioxidative and antiapoptotic actions of L- carnitine that demonstrated in several tissues (30, 31), could also take place in bone tissue.

In this study, body weight of the rats increased steadily throughout the study. As was seen in previous studies, ovariectomized rats had significantly higher body weight compared to sham-operated rats due to fat deposition caused by estrogen deficiency (9, 32). The effects of ovariectomy on weight gain have long been established. Ovariectomized rats had increased food intake as their appetite was increased due to low levels of leptin released by adipose tissue associated with estrogen deficiency. Leptin works on hypothalamus to control food intake and energy expenditure and its deficiency will leads to increased appetite and food intake (33). This research observed that daily α-tocopherol administration in OVX+AT group was failed to prevent the weight gain induced by ovariectomy. However, L-carnitine administration both in OVX+C and OVX+C+AT groups was able to prevent the weight gain associated with ovariectomy. This result agrees with others studies that demonstrated a significant role of carnitine on reducing obesity, food intake, and high serum leptin level caused by high-fat diet in C57BL/6J mice (34, 35).

The main function of carnitine in the body is facilitation lipid oxidation by transporting long-chain fatty acids into the inner mitochondria region where they undergo β-oxidation. Prior to β-oxidation fatty acids must be changed into acyl CoA which cannot cross the mitochondrial membrane. Carnitine acts as a cofactor in beta-oxidation by facilitating acylCoA entrance into mitochondria in the form of acylcarnitine esters through carnitine-palmitoyl transferase system (CPT-I and II) and the exit of acetyl groups from mitochondria to the cytosol through carnitine acyl transferase system (36). Therefore, without carnitine, most of the dietary lipids cannot be used as an energy source and our body would accumulate fatty-acids resulting in obesity.

In this study, we also managed to demonstrate the bone biochemical marker changes characteristics of osteoporosis. ALP and osteocalcin are expressed and synthesized by mature osteoblast and are generally regarded as a specific marker for osteoblastic activity and for bone formation. While, CTX are peptide fragments from collagen degradation that is released by osteoclasts during bone resorption and are considered as a specific marker for osteoclastic activity and bone resorption (37). Consistent with other researches (8,38), the results of this study revealed a significant increase in both bone formation and resorption markers in ovariectomized rats as compared to rats of sham group. The mechanism for bone loss is a function of the negative imbalance of bone resorption and formation. Soon after the OVX procedure, 400 and 270% rate increases in bone resorption and formation occurs respectively and consequently a detectable bone loss appeared on the 14th day (39) with increased in both bone formation and resorption markers.

Treatment with α-tocopherol showed significant decrease in serum CTX and significant increase in serum ALP with insignificant change in osteocalcin levels as compared to OVX group. Despite these favorable changes in formation and resorption markers observed in this study, α-tocopherol at this point in time had not reversed OVX induced alternation in BMD, BMC, CBT, TBD, and mean TBA. While, treatment with L-carnitine showed more significant increase in serum ALP, osteocalcin, and decrease in CTX and inorganic phosphorus levels as compared to OVX and the group of rats treated with α-tocopherol.

Moreover, the present results revealed that the group of rats receiving combined treatment with L- carnitine and α-tocopherol showed higher bone mass and more improvement in bone remodeling markers (ALP, osteocalcin, and CTX) than those receiving L-carnitine alone. Since the combined treatment of L-carnitine and α-tocopherol increased bone parameters more than the cumulative effects of α-tocopherol alone plus L- carnitine alone, these observations suggest that L-carnitine and α-tocopherol had synergistic effects on the skeleton. These beneficial effects of α-tocopherol in association with L- carnitine may be mediated via its antioxidant activity. Several reports have suggested that oxidative stress play an important role in the development of post-menopausal osteoporosis (40, 41). Also, estrogen deficiency, which is the main cause of osteoporosis in post-menopausal women, is associated with increasing bone resorbing factors such as interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), prostaglandine E2 (PGE2) and receptor activator of nuclear factor kappa-B ligand (RANKL), while decreasing osteoclastogenesis-inhibiting factors such as osteoprotegerin (OPG) (42). This leads to an increase in activity and number of osteoclasts compared to osteoblast activity, resulting in an overall loss of bone. The positive effects of α-tocopherol on bone by preventing oxidative stress could be mediated via similar pathway involving the RANK/RANKL. Ha et al. (43) showed that Vitamin E prevented osteoclastogenesis and bone resorption by suppressing RANKL expression and signalling without affecting OPG expression.

In conclusion, the results of this study demonstrate that supplementation with α-tocopherol was unable to reverse the loss of bone due to ovarian hormone deficiency. On the other hand, treatment with L-carnitine was able to restore the loss of BMD and BMC and reverse the decrease in CBT and mean TBD in the same animal model of osteoporosis. Moreover, supplementation of L-carnitine in combination with α-tocopherol at clinically acceptable doses showed more improvement in bone mass and michroarchitecture compared to L-carnitine or α-tocopherol alone. Therefore, the combination of L-carnitine and α-tocopherol has potential for the treatment of post-menopausal osteoporosis.

Abbreviations: CTX, cross- linked c-terminal telopeptide; ALP, alkaline phosphatase; BMD, bone mineral density; BMC, bone mineral content; CBT, mean cortical bone thickness; TBD, mean trabecular bone density.

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