Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

L-Carnitine, but not coenzyme Q10, enhances the anti-osteoporotic effect of atorvastatin in ovariectomized rats

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Received Mar. 17, 2015; Revision accepted Sept. 14, 2015; Crosschecked Dec. 10, 2015

Abstract: Objective: Statins' therapy in osteoporosis can aggravate muscle damage. This study was designed to assess which agent, L-carnitine or coenzyme Q10, could enhance the anti-osteoporotic effect of atorvastatin while antagonizing myopathy in ovariectomized rats. Methods: Forty-eight female Sprague Dawley rats were used; forty rats were ovariectomized while eight were sham-operated. Eight weeks post-ovariectomy, rats were divided into ovariectomized-untreated group and four ovariectomized-treated groups (n=8) which received by gavage (mg/(kg·d), for 8 weeks) 17β-estradiol (0.1), atorvastatin (50), atorvastatin (50)+L-carnitine (100), or atorvastatin (50)+coenzyme Q10 (20). At the end of therapy, bone mineral density (BMD), bone mineral content (BMC), and serum levels of bone metabolic markers (BMMs) and creatine kinase (CK) were measured. Femurs were used for studying the breaking strength and histopathological changes. Results: Treatment with atorvastatin+L-carnitine restored BMD, BMC, and bone strength to near normal levels. Estrogen therapy restored BMD and BMC to near normal levels, but failed to increase bone strength. Although atorvastatin and atorvastatin+coenzyme Q10 improved BMD, BMC, and bone strength, they failed to restore levels to normal. All treatments decreased BMMs and improved histopathological changes maximally with atorvastatin+L-carnitine which restored levels to near normal. Atorvastatin aggravated the ovariectomy-induced increase in CK level while estrogen, atorvastatin+L-carnitine, and atorvastatin+coenzyme Q10 decreased its level mainly with atorvastatin+L-carnitine which restored the level to near normal. Conclusions: Co-administration of L-carnitine, but not coenzyme Q10, enhances the anti-osteoporotic effect of atorvastatin while antagonizing myopathy in ovariectomized rats. This could be valuable in treatment of osteoporotic patients. However, further confirmatory studies are needed.

Key words: Atorvastatin, Coenzyme Q10, L-Carnitine, Ovariectomized
http://dx.doi.org/10.1631/jzus.B1500065 **CLC number:** R969.2

1 Introduction

The bone loss in osteoporosis decreases bone mineral density (BMD), bone quality, and bone strength. The resultant increased risk of fracture is the central cause of morbidity and mortality in osteoporosis. Anti-resorptive therapy of osteoporosis decreases fracture risk and this is partly explained by an increase in BMD (Cummings *et al*., 2002). Bone quality is determined by bone mass, size, structure, and remodeling. It is badly affected by high remodeling or turnover rate. Remodeling starts with bone resorption by osteoclasts followed by bone formation by osteoblasts. It is assessed by measurement of bone metabolic markers (BMMs) of bone formation, such as bone alkaline phosphatase (BALP) and osteoprotegerin (OPG), and that of bone resorption such as type I collagen C-telopeptide (CTX-1) (Parfitt, 2002; Krakauer, 2008; Bouzid *et al*., 2010). In healthy individuals, bone resorption and formation are balanced while in an estrogen deficiency milieu,

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such as in postmenopausal women, there is increased bone loss which may be due to oxidative stress created by increased hydrogen peroxide and lipid peroxidation levels and low antioxidant enzyme activity (Muthusami *et al*., 2005). In addition, estrogen deficiency causes damage of skeletal muscle and leakage of creatine kinase (CK) increasing its serum level (Amelink *et al*., 1990). Osteoporosis affects trabecular bone in metaphysis of long bone in animals and human. The histopathological changes in femurs of the postmenopausal osteoporotic women were found to be similar to those in the ovariectomized (OVX) rats. Thus, the OVX rat is considered to be a "gold standard experimental model" for testing treatments for postmenopausal osteoporosis (Kharode *et al*., 2008).

The best clinical assessment of fracture risk in osteoporosis is achieved by measurements of both BMD and BBMs. In addition, BBMs play a role in individualizing therapy for osteoporotic patients especially in severe cases. Patients with high markers are preferably treated with drugs that reduce bone turnover while those with low markers are preferably treated with anabolic treatments. BBMs are also used to assess effectiveness of treatment within 3–6 months in contrast to changes in BMD that can only be detected after about 2 years. However, this approach is still controversial (Writing Group for the ISCD Position Development Conference, 2004). BALP is released into the serum from membranes of osteoblasts upon their activation. It helps mineralization of the bone matrix through elevating local levels of inorganic phosphates (Brown *et al*., 2009). On the other hand, activation of osteocalsts cleaves CTX-1 from type I collagen, which is the most essential organic component of the osteoid matrix, and thus CTX-1 is considered a good marker for bone turnover (Bouzid *et al*., 2010). The receptor activator of nuclear factor kappa-B (RANK) is bound to the osteoclasts' surface while its ligand (RANKL) is bound to the osteoblasts' surface. Binding of RANKL to RANK can activate the three MAPK (mitogen-activated protein kinase) pathways and the NF-κB (nuclear factor kappa B) pathway and triggers osteoclastic differentiation and activity. OPG, a cytokine receptor produced by osteoblasts, is a member of the tumor necrosis factor (TNF) receptor family. By binding RANKL, OPG inhibits RANK activation and the subsequent activation of NF-κB (Krakauer, 2008).

There is "a link between osteoporosis and cardiovascular disease that cannot be explained by age alone" (Farhat and Cauley, 2008). Statins, the anti-hypercholesterolemic agents, offer a double benefit for patients having both osteoporosis and cardiovascular problems. Statins decrease synthesis of cholesterol through inhibition of the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (Endo, 1992) at early stages and therefore they also reduce formation of ubiquinone (coenzyme Q10, CoQ10) and other metabolites needed for muscle cell functions. Statin-induced myotoxicity occurs mainly due to mitochondrial impairment and oxidative stress (Sirvent *et al*., 2008; Taha *et al*., 2014). Statins improve osteoporosis through cholesterol-dependent and -independent (pleiotropic) effects and atorvastatin is the most effective in this respect possibly due to its lipophilic property (Nakahara *et al*., 1994; Staal *et al*., 2003). In 10% of patients, statins, especially in high doses, cause myopathy, with or without increased serum CK level, which may result in renal failure, hepatic dysfunction, or pancreatitis (Venero and Thompson, 2009). Statins' myopathy may be due to carnitine abnormalities or decreased CoQ10 levels and thus L-carnitine and CoQ10, acting as radical scavengers, can prevent oxidative mitochondrial damage and protect muscles.

Statins' toxicity is postulated to be mainly due to mitochondrial oxidative stress and inhibition of mitochondrial respiration. L-Carnitine, besides its metabolic role, has an antioxidant effect and can directly decrease superoxide radical generation. CoQ10 is an essential electron transporter in the respiratory chain and its reduced form (ubiquinol) shows antioxidant properties (Young *et al*., 2011; Bookstaver *et al*., 2012; La Guardia *et al*., 2013). Simvastatin increases cytoplasmic Ca^{2+} triggering mitochondrial reactive oxygen generation and myotoxicity (Sirvent *et al*., 2005a). In the isolated rat skeletal muscle mitochondria, both atorvastatin and simvastatin inhibited respiration and β-oxidation (Kaufmann *et al*., 2006). In human skeletal myotubes, simvastatin caused mitochondrial respiratory dysfunction and oxidative stress (Kwak *et al*., 2012). The toxic dose of simvastatin caused death of the prostate cancer-3 cell line through increasing cytosolic Ca^{2+} level and

causing oxidative mitochondrial damage (Costa *et al*., 2013). Simvastatin-induced mitochondrial dysfunction results from suppression of complex I of the respiratory chain. This mechanism is specific for skeletal muscles partly due to uptake of statins by a monocarboxylate transporter which is not expressed in cardiac myocytes (Sirvent *et al*., 2005b). Statins caused hepatotoxicity in rats through increasing free radical formation, lipid peroxidation, and mitochondrial depolarization in addition to decreasing the cellular glutathione reservoirs (Abdoli *et al*., 2013).

This study was designed to assess which agent, L-carnitine or CoQ10, could enhance the anti-osteoporotic effect of atorvastatin while protecting against myopathy in the OVX rats.

2 Materials and methods

2.1 Ovariectomy and experimental design

This study was reviewed and approved by the Institutional Research Ethics Committee and adhered to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals. Forty-eight 6-month-old female Sprague Dawley rats ((180±20) g) were used. After a week of acclimatization, 40 rats were subjected to an ovariectomy operation. Under anesthesia with diethyl ether, an incision of about 5 mm was made in each of the dorsal flank regions of the rat to expose ovaries. The fallopian tubes were sutured at the most distal ends and ovaries were excised. The other eight rats were subjected to the same procedure without excising ovaries to serve as sham non-OVX (NOVX) control. The incisions were sutured and dressed with povidone iodine for 7 d (Reddy Nagareddy and Lakshmana, 2005). Eight weeks post-surgery, BMD and bone mineral content (BMC) were measured in all OVX rats to confirm occurrence of osteoporosis.

The OVX rats were randomly divided into five groups (n=8) treated daily for eight weeks by oral gavage as follows: OVX-untreated (saline), OVX+E2 (17β-estradiol, 0.1 mg/kg (Huber *et al*., 2007)), OVX+AT (atorvastatin, 50 mg/kg (Uyar *et al*., 2009; Chang *et al*., 2011)), OVX+AT+LC (atorvastatin+L-carnitine, 100 mg/kg (Moustafa and Boshra, 2011)), and OVX+AT+CQ (atorvastatin+CoQ10, 20 mg/kg (Chirapapaisan *et al*., 2012)). At the end of treatment, BMD and BMC were measured. Later, rats were anesthetized by diethyl ether and sacrificed by cervical dislocation. Blood was collected by cardiac puncture for biochemical measurements. The femurs were excised, wrapped in saline-moistened gauze, and frozen at −70 °C and then the right femur was used for histological examination and left femur for assessment of breaking strength (Bitto *et al*., 2009). Drugs and chemicals were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA) unless otherwise stated.

2.2 BMD and BMC

Measurements were made by scanning the whole rat body using dual X-ray absorptiometry (DXA; Lunar Prodigy, General Electric, Madison, WI, USA) with software version 1.45 for small animals. This method is useful for studying the whole skeleton and individual long bones. Rats were anaesthetized with intraperitoneal sodium pentobarbital (40 mg/kg), and then placed in a prone position in middle of the measurement table. All rats were scanned for 30 min by the same technician to minimize mistakes. BMC and BMD were expressed in g and g/cm^2, respectively (Karahan *et al*., 2002).

2.3 Femur breaking strength

Femur breaking strength was measured by a three-point bending test using the hardness tester (Erweka GmbH, Heusenstamm, Germany) as previously described by Ko *et al.* (2012) with minor modification by Abdallah *et al.* (2014). The length of left femur was measured, its center was marked, and then it was placed in the clamp assembly of the tester in a position horizontal to the force direction. The load was applied at center of femur at a rate of 10 mm/min until fractured and the maximum load tolerated by it was expressed in Newtons (N).

2.4 Histological examination

The right femur was fixed in 10% neutral buffered formalin for 24 h at 37 °C, dehydrated in 95% ethanol, and then embedded in paraffin. Horizontal sections (3–5 μm thick) were cut, stained with hematoxylin and eosin (H&E) and then examined using light microscopy (Bitto *et al*., 2009).

2.5 BMMs

Commercially available rat ELISA kits were used for measurement of serum levels of BALP (IDS Ltd., UK) (Bitto *et al*., 2009), OPG (Immundiagnostik AG, Bensheim, Germany) (Bae and Kim, 2010), and CTX-1 (Nordic Bioscience Diagnostics A/S, Herlev, Denmark) (Høegh-Andersen *et al*., 2004).

2.6 Skeletal muscle damage marker

Serum CK level was determined using a commercially available kit (CK Liqui UV Test, Stanbio Laboratory, Boerne, TX, USA) (Siekmann *et al*., 2002).

2.7 Statistical analysis

Data were expressed as means±standard errors of the means (SEM) and analyzed using SPSS 18. Comparisons for two groups were made using the Student's *t*-test. One-way analysis of variance (ANOVA) with Tukey's test was used for comparison of more than two groups. A P value of <0.05 was considered to be statistically significant.

3 Results

3.1 Body weight (BW)

The final BWs significantly increased compared to the initial ones and ovariectomy significantly increased BW compared to NOVX rats. The OVX-treated groups showed non-significant changes compared to the OVX group (Table 1).

Table 1 Effect of treatments (for eight weeks) on body weight (BW) in OVX rats

Treatment	Initial BW (g)	Final BW (g)
NOVX	182.13±2.00	202.50±2.06
OVX	179.50±3.04	213.13±2.44*
OVX+E2	183.25±1.84	215.38±1.94*
OVX+AT	180.88±2.14	212.50±1.96*
OVX+AT+LC	178.25±1.62	212.75±1.74*
OVX+AT+CQ	185.38±2.07	214.75±2.88*

NOVX: non-ovariectomized; OVX: ovariectomized; E2: 17β-estradiol (0.1 mg/(kg·d)); AT: atorvastatin (50 mg/(kg·d)); LC: L-carnitine (100 mg/(kg·d)); CQ: coenzyme Q10 (20 mg/(kg·d)). * A significant difference (P<0.05) vs. NOVX rats. Data are expressed as mean±SEM (n=8)

3.2 BMD and BMC

The initial parameters, BMD and BMC, eight weeks after ovariectomy, significantly decreased compared to NOVX rats confirming the occurrence of osteoporosis. The final parameters after 8-week therapy, showed significant increases compared to OVX rats. The OVX+AT+LC group showed more significant increases than OVX+AT and OVX+AT+CQ groups. Both OVX+E2 and OVX+AT+LC treatments restored the normal levels with non-significant difference from the NOVX group, while OVX+AT and OVX+AT+CQ groups still showed significantly lower values (Figs. 1a and 1b).

3.3 Femur breaking strength

The OVX rats showed significantly decreased maximal load values compared to NOVX rats. All treatments except E2 significantly reversed these changes, indicating failure of E2 to restore bone strength. The OVX+AT+LC treatment restored bone strength to near normal with a non-significant difference from the NOVX rats while the OVX+AT and OVX+AT+CQ groups still showed significantly lower values (Fig. 1c).

3.4 Histological examination

The femurs of NOVX rats showed intact and well-formed trabeculae with normal trabecular spaces. The OVX group showed a significantly decreased trabecular number and widened trabecular spaces. All treatments significantly improved these changes, most significantly with OVX+AT+LC treatment which showed a non-significant difference from the NOVX group (Figs. 1d and 2).

3.5 BMMs

The OVX group showed significantly increased markers (BALP, OPG, and CTX-1) confirming increased bone turnover compared to NOVX rats. All treatments significantly decreased the markers compared to OVX rats. The decrease was most significant with the OVX+AT+LC group restoring levels to near normal with non-significant differences from the NOVX group (Figs. 3a–3c).

3.6 Skeletal muscle damage marker

The OVX group showed a significantly increased CK level, indicating myopathy compared to NOVX rats. Atorvastatin further increased the CK level compared to OVX rats, indicating aggravation of myopathy, while E2 therapy and addition of LC or

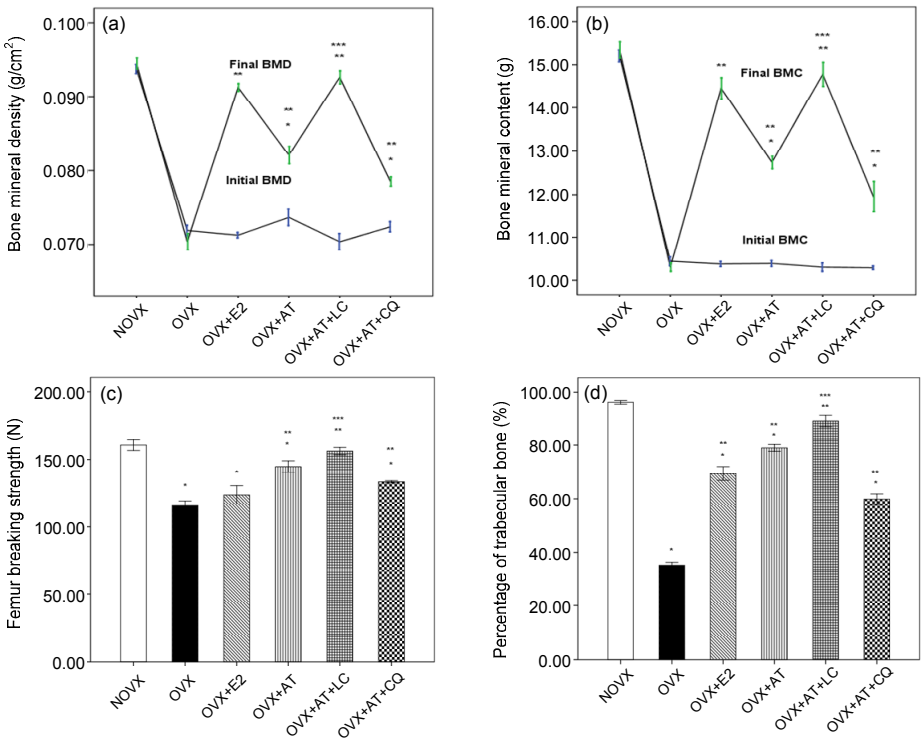

Fig. 1 Effects of treatments (for eight weeks) on bone mineral density (BMD) (a), bone mineral content (BMC) (b), femur breaking strength (c), and percentage of trabecular bone (d)

NOVX: non-ovariectomized; OVX: ovariectomized; E2: 17β-estradiol (0.1 mg/(kg·d)); AT: atorvastatin (50 mg/(kg·d)); LC: L-carnitine (100 mg/(kg·d)); CQ: coenzyme Q10 (20 mg/(kg·d)). * A significant difference (P<0.05) vs. NOVX; ** A significant difference (P<0.05) vs. OVX; *** A significant difference (P<0.05) for OVX+AT+LC vs. OVX+AT and OVX+AT+CQ (a–d) and also vs. OVX+E2 (d). Data are expressed as mean±SEM (n=8)

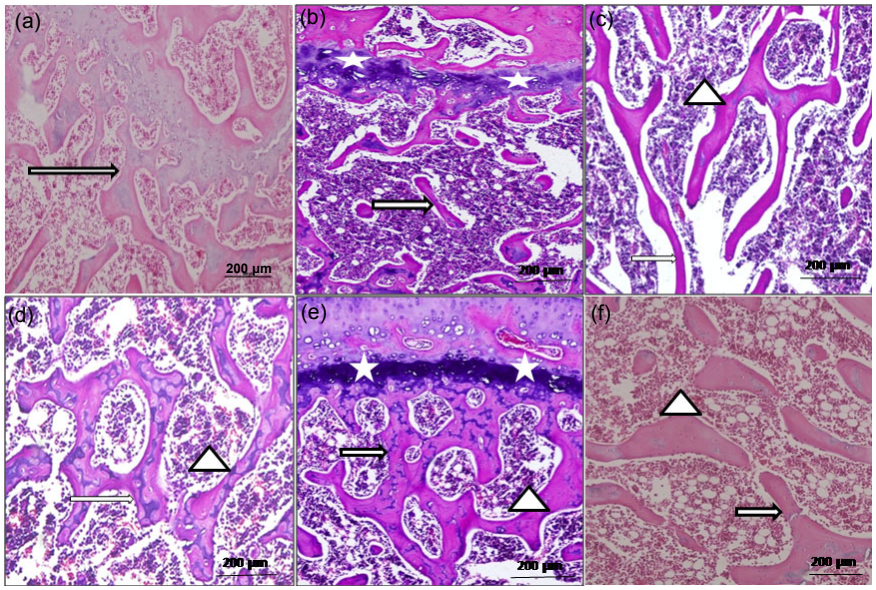

Fig. 2 Femurs in different rat groups stained with hematoxylin and eosin

(a) NOVX (non-ovariectomized) rats showed intact and well-formed trabeculae (white arrow) with normal marrow spaces. (b) OVX (ovariectomized) rats showed an irregular and markedly deformed epiphyseal plate (white stars), decreased trabecular thickness (white arrow), and widened marrow spaces. (c, d, e, f) The treated groups with 17β-estradiol, atorvastatin, atorvastatin+L-carnitine, and atorvastatin+coenzyme Q10, respectively, showed increases in trabecular thickness (white arrows) and narrowing of the widened spaces with newly formed osteoid tissue (white triangles) maximally in the atorvastatin+L-carnitine group which is nearly normal with a regular epiphyseal plate (white stars)

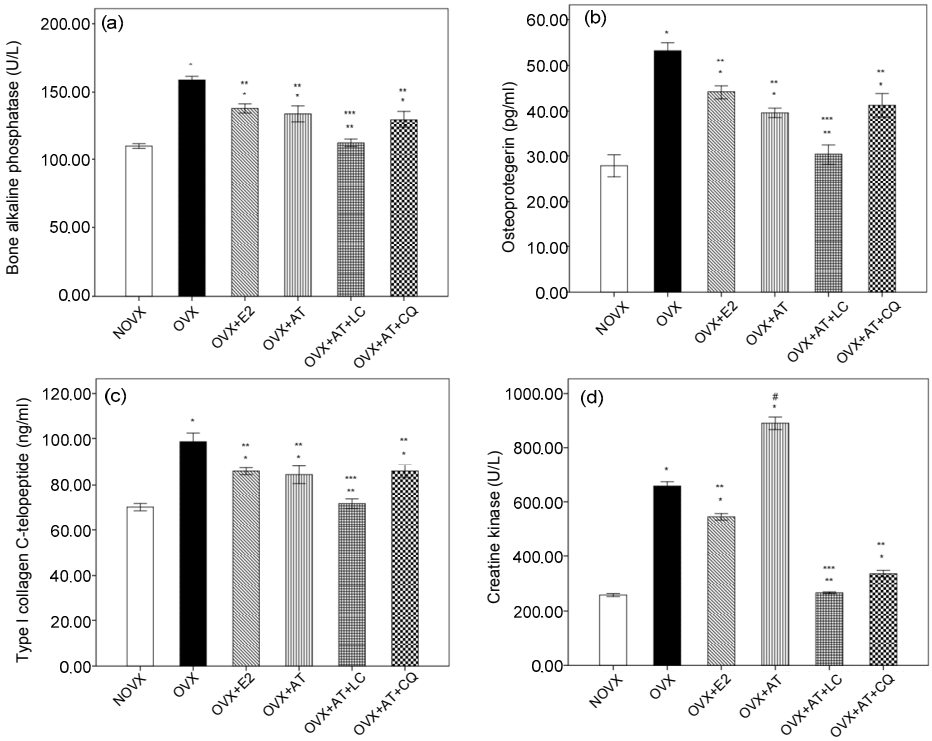

Fig. 3 Effects of treatments (for eight weeks) on serum levels of bone alkaline phosphatase (BALP) (a), osteoprotegerin (OPG) (b), type I collagen C-telopeptide (CTX-1) (c), and creatine kinase (CK) (d)

NOVX: non-ovariectomized; OVX: ovariectomized; E2: 17β-estradiol (0.1 mg/(kg·d)); AT: atorvastatin (50 mg/(kg·d)); LC: L-carnitine (100 mg/(kg·d)); CQ: coenzyme Q10 (20 mg/(kg·d)). * A significant difference (P<0.05) vs. NOVX; ** A significant difference (P<0.05) vs. OVX; *** A significant difference (P<0.05) for OVX+AT+LC vs. other treatments; # A significant increase (P<0.05) vs. OVX. Data are expressed as mean±SEM (n=8)

CQ to atorvastatin significantly decreased CK levels. The decrease was most significant in the OVX+AT+LC group restoring the level to near normal with a non-significant difference from the NOVX group (Fig. 3d).

4 Discussion

Drugs used in the treatment of osteoporosis, such as estrogen, teriparatide, bisphosphonates, statins, and selective estrogen receptor modulators, have antiresorptive and/or bone-forming effects (Seeman *et al*., 2008). The ovariectomy in rats, eight weeks post-surgery, decreased BMD, BMC, and femur strength, and increased serum levels of BALP, OPG, and CTX-1. In addition, it decreased trabecular number and thickness with widening of trabecular spaces in the cancellous femur. After 8-week therapy, a combination of atorvastatin and L-carnitine succeeded in restoring the levels of BMD, BMC, and bone strength to near normal. While estrogen therapy restored the levels of BMD and BMC to near normal, it failed to increase bone strength. Atorvastatin alone or plus CoQ10 improved the levels of BMD, BMC, and bone strength, but failed to restore them to normal. All treatments improved the histopathological changes and decreased the BMMs mainly with the combination of atorvastatin and L-carnitine which restored the changes to near normal.

In line with the current results, it was shown that estrogen therapy reversed the ovariectomy-induced increase of serum CTX-1 level back to near normal. The OVX rats also showed higher OPG and RANKL levels, indicating increased bone turnover (Bae and Kim, 2010). In addition, at 8 weeks post-ovariectomy, serum BALP level increased by 38.7% and OPG level showed a similar pattern (Miyazaki *et al*., 2004). The RANKL/OPG pathway is shared by statins, osteoporosis and adipogenesis (Tsartsalis *et al*., 2012). A significant decrease in trabecular number with widening of the trabecular spaces occurred in the OVX rats' femur 6 weeks post-ovariectomy

(Estai *et al*., 2011). The decrease in cholesterol levels affects the Akt-mTOR (protein kinase B-mammalian target of rapamycin) signaling and stimulates osteoclast apoptosis (Luegmayr *et al*., 2004). The ovariectomy-induced increase in bone turnover is manifest by significant increases of serum levels of ALP (a marker of bone formation) (Seif, 2014) and acid phosphatase (ACP, a marker of bone resorption). Estrogen therapy significantly decreased ACP level to near 17.2% of its level in the OVX group (Abdallah *et al*., 2014). There is a link between strength of femur neck and BMC in rats and thus fracture risk in osteoporosis is closely related to quality of the cortical bone (Nakamura *et al*., 2008).

In rats, bone remodeling and modeling happen mainly in areas of cancellous and cortical bone respectively resulting in decreased or increased bone mass. While the anti-resorptive agents affect mainly the bone remodeling area, the anabolic agents, such as statins, affect both areas. A decrease in BMD and BMC does not necessarily mean a similar decrease in bone strength, thus direct testing of the bone mechanical properties is important because they are directly related to fracture risk. Decreased bone strength can occur due to altered distribution of bone trabeculae without actual bone loss. Also rapid bone turnover may not allow complete mineralization, causing decreased bone mass and strength due to weak trabeculae (Ott, 2001). The load values needed to break the femur in the horizontal and vertical directions significantly decreased in OVX rats to 34% and 56% of that needed for a normal femur, respectively. Estrogen (10 mg/kg) did not affect bone strength in the horizontal direction but significantly improved it in the vertical direction by 89.3% (Abdallah *et al*., 2014). In OVX rats, atorvastatin prevented the OVX-induced decreases in BMD and bone strength, improved bone hardness by about 43.3% restoring the original horizontal bone hardness, and repaired structure of both cortical and trabecular bone matrices in the head of the femur (Uyar *et al.*, 2009). Orchidectomy decreased both BMD and load values of left femurs and increased serum levels of BALP, OPG, and CTX-1 compared to non-orchidectomized rats. Atorvastatin treatment (12 mg/(kg·d)) for 12 weeks reversed these orchidectomy-induced changes (Gradosova *et al*., 2012). Atorvastatin increased production of OPG in human osteoblasts (Viereck *et al*., 2005), and decreased serum N-terminal telopeptide of type I collagen (a marker of bone resorption) in hypercholesterolemic male patients (Majima *et al*., 2007). In contrast, statins failed to prevent the OVX-induced bone loss in rats. These controversial results may be due to different study designs and use of different statins (Yao *et al*., 2006).

In OVX rats, estrogen deficiency caused a leakage of CK from the skeletal muscles, increasing its serum level. While atorvastatin further aggravated this myopathy, estrogen and supplementation of atorvastatin with L-carnitine or CoQ10 decreased CK levels mainly with the combination of atorvastatin and L-carnitine which restored the CK level to near normal. Estrogen protects skeletal muscles through its antioxidant effect (Sotiriadou *et al*., 2003). Statins' myopathy, mainly postulated to cause oxidative damage of the mitochondrial respiratory chains, is reversed by L-carnitine and CoQ10 through their direct radical scavenging effects (La Guardia *et al*., 2013). Simvastatin's myopathy impairs phosphatidylinositol 3-kinase/Akt signaling in rodent skeletal muscle (Mallinson *et al*., 2012).

The protective effects of L-carnitine have been reported in multiple studies. L-Carnitine is a natural endogenous quaternary ammonium compound that plays an essential role in the mitochondrial transport and oxidation of fatty acids. Factors that affect its levels possibly affect ATP levels. L-Carnitine and acetyl-L-carnitine participate in "control of the mitochondrial acyl-CoA/CoA ratio, peroxisomal oxidation of fatty acids, and production of ketone bodies." L-Carnitine exerts neuroprotective effects against many neurotoxic conditions resulting from mitochondrial dysfunction (Virmani *et al*., 2005). The antioxidant activity of L-carnitine was evaluated and it was found to have an effective antioxidant property (Gülçin, 2006). L-Carnitine protects against simvastatin-induced cell necrosis in prostate cancer-3 cell line through decreasing the simvastatin-induced mitochondrial overproduction of the superoxide anion and preventing oxidative mitochondrial dysfunction (Costa *et al*., 2013). L-Carnitine stimulates mitochondrial β-oxidation preventing free fatty acid-induced oxidative stress and reducing hepatotoxicity (Jun *et al*., 2011).

The correction of atorvastatin-induced myopathy by co-administration of L-carnitine in rats was

manifested by a non-significant increase in CK and improvement of histological changes (Mazroa and Asker, 2010). In OVX rats, oral L-carnitine (100 mg/(kg·d) for two months) significantly reduced skeletal muscle degeneration (Moustafa and Boshra, 2011). Also it decreased the CK level, improved skeletal muscle histopathological changes, and improved mitochondrial respiratory function (Kumaran *et al*., 2005). It was found that L-carnitine assists transference of long-chain fatty acids into mitochondria for β-oxidation and thus regulates the quantity of energy available for protein synthesis in osteoblasts (Patano *et al*., 2008). Moreover, L-carnitine increased osteoblast activity and reduced bone loss in the elderly (Colucci *et al*., 2005). In aged OVX rats, dietary L-carnitine supplementation for eight weeks did not affect food intake, body weight, or cholesterol level. It significantly decreased triglycerides, ACP, and ALP, and improved bone microstructure by decreasing bone turnover (Hooshmand *et al*., 2008). In OVX rats, L-carnitine decreased muscle damage suggesting a potential protective effect against postmenopausal myopathy (Moustafa and Boshra, 2011). The current evidence does not support CoQ10 supplementation in statins' myopathy (Schaars and Stalenhoef, 2008). However, Zlatohlavek *et al.* (2012) found that administration of CoQ10 for six months significantly decreased statin-induced muscle pain. Moreover, CoQ10 inhibited RANKL-induced osteoclast activation and stimulated osteoblasts, suggesting its potential use in treatment of osteoporosis (Moon *et al*., 2013).

Finally, this work is considered to be an addition to previous studies that attempted to find novel approaches for treatment of osteoporosis and its comorbidities. Recently, L-carnitine was found to stimulate endothelial nitric oxide synthase (eNOS), increasing production of NO (Ning and Zhao, 2013). The increased NO has been found to influence BMD and increase serum estradiol level, protecting against osteoporosis (Liu *et al*., 2009). In a meta-analysis of 13 controlled trials (3629 subjects), L-carnitine improved the metabolic competence and mortality in chronic heart failure (Wang and Guo, 2013). In another meta-analysis of 22 studies (1492 subjects), it was found that antiepileptic drugs reduced BMD and enhanced risk of fracture in epileptic children (Zhang *et al*., 2015). Statins, through enhancing the reduced BMD, could be beneficial for such children. Also co-administration of statins was found to help regulate seizure predisposition in mice (Russo *et al*., 2013). In addition, sodium ferulate (a Chinese traditional medicine) was found to reduce incidence of the steroid-induced necrosis of the head of femur in rabbits (Tian *et al*., 2013).

5 Conclusions

Co-administration of L-carnitine, but not CoQ10, enhances the anti-osteoporotic effect of atorvastatin while antagonizing myopathy occurring as a complication of both atorvastatin therapy and ovariectomy in rats. If the present findings can be translated to clinical reality, they could be valuable in the treatment of osteoporotic patients. However, this translation needs further confirmatory studies.

Compliance with ethics guidelines

Hussam A. S. MURAD declares that he has no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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中文概要

题　目：左卡尼汀（而非辅酶 Q10）提高阿托伐他汀在切除卵巢的老鼠的抗骨质疏松作用

目　的：他汀类药物在治疗骨质疏松症时会加重肌肉损伤。本实验研究左卡尼汀和辅酶 Q10 对阿托伐他汀在切除卵巢的老鼠的抗骨质疏松作用的影响。

创新点：研究新的治疗骨质疏松症及其并发症的方法。

方　法：选取 48 只雌性 SD 大鼠，40 只大鼠切除卵巢，8 只为假手术组。切除卵巢 8 周后，大鼠被分成去卵巢非治疗组和 4 个去卵巢治疗组（每组 8 只），通过灌胃法给药（单位为 mg/(kg·d)，为期 8 周）：雌二醇（0.1）、阿托伐他汀（50）、阿托伐他汀（50）+左卡尼汀（100）或阿托伐他汀（50）+辅酶 Q10（20）。在治疗结束时，测量骨矿物质密度、骨矿物质含量及骨代谢标志物和肌酸激酶的血清水平，利用股骨研究抗断强度和组织病理学变化。

结　论：相比辅酶 Q10，合并给药左卡尼汀可在提高阿托伐他汀对切除卵巢的老鼠的抗骨质疏松作用的同时避免肌肉损伤。

关键词：阿托伐他汀；辅酶 Q10；左卡尼汀；切除卵巢

（感谢山东大学药学院常明露提供中文概要）