Oral supplementation with antioxidant agents containing alpha lipoic acid: effects on postmenopausal bone mass

G. Mainini[1], M. Rotondi[2], K. Di Nola[3], M.T. Pezzella[3], S.A. Iervolino[3], E. Seguino[3], D. D'Eufemia[3], I. Iannicelli[3], M. Torella[3]

[1]"San Leonardo" Hospital, Castellammare di Stabia, Naples; [2]Azienda Sanitaria Locale, Salerno
[3]Department of Gynecology, Obstetrics and Reproduction, Second University of Naples (S.U.N.), Naples (Italy)

Summary

Purpose of investigation: Oxidative stress impacts many age-related degenerative processes, such as in postmenopausal bone loss and in antioxidant defenses that are significantly decreased in elderly osteoporotic women. The authors evaluated the effect of oral supplementation with antioxidant agents containing alpha lipoic acid (ALA) on bone mineral density (BMD) of osteopenic postmenopausal women. *Materials and Methods:* Fifty postmenopausal women with osteopenia (– 2.5 < T-score < – 1) were prospectively enrolled and randomly assigned to orally receive ALA and other antioxidant agents (vitamin C, vitamin E, and selenium) plus calcium and vitamin D3 (n = 25), or only calcium and vitamin D3 (n = 25). The BMD was estimated at baseline and after 12 months of treatment by heel quantitative ultrasonometry (QUS). *Results:* Forty-four patients completed the one-year study: 23 in the ALA group, 21 in the control group. The treatment of ALA group led to a better estimated BMD compared to the control group (0.401 ± 0.026 vs 0.388 ± 0.025 g/cm^2), although this difference barely achieved a statistical significance (p = 0.048). *Conclusion:* These findings, although in a small population, could suggest that oral supplementation with antioxidant agents containing ALA may mitigate bone loss in osteopenic postmenopausal women.

Key words: Alpha lipoic acid; Menopause; Osteoporosis.

Introduction

Alpha lipoic acid (ALA), or thioctic acid, is a vitamin-like fatty acid produced by the human organism and found in small amounts in several foods: muscle meats, heart, kidney, liver and, to a lesser degree, fruits and vegetables.

ALA is involved in the Krebs cycle and plays a key-role in the cellular energetic metabolism with an insulin-like stimulation of glucose uptake and utilization [1]. In particular, ALA is a naturally occurring cofactor for the mitochondrial enzymes pyruvate-dehydrogenase and alpha-ketoglutarate-dehydrogenase, then it increases acetylcholine (ACh) production by activation of choline-acetyltransferase and increases glucose uptake, thus supplying more acetyl-CoA for the production of ACh [2].

Moreover, ALA is also a powerful antioxidizing agent [3-5]. In particular, ALA chelates redox-active transition metals, thus inhibiting the formation of hydroxyl radicals and also scavenges reactive oxygen species (ROS), thereby increasing the levels of reduced glutathione [6-11]. In addition, ALA down-regulates the expression of redox-sensitive pro-inflammatory proteins, including tumor necrosis factor (TNF) and inducible nitric oxide synthase (iNOS) [12, 13]. Furthermore, ALA can scavenge lipid peroxidation products, such as hydroxynonenal and acrolein [14, 15].

Oxidative stress plays a pivotal role in the pathogenesis of various diseases and many age-related degenerative processes, including aging, cancer, atherosclerosis, inflammation, diabetes, and Parkinson's disease [16]. Many studies have been conducted confirming the clinical benefits of ALA, including recent findings that ALA offers enhancing effects on hypertension, coronary heart disease, metabolic syndrome, peripheral neuropathy including diabetic neuropathy, and brain function including Alzheimer's disease [17]. Clinically, ALA has been widely used for long time, including in ischemia reperfusion injury [18], diabetic neuropathy [19], HIV infection [20], and neurodegenerative diseases [21].

Furthermore, several sources of evidence have suggested a possible link between oxidative stress and bone loss: directly, by osteoclast-generated superoxide contributing to bone degradation, and indirectly, by induction of the osteoclast differentiation [22-24]. Moreover, some antioxidant defenses (e.g., vitamins C, E, and selenium) are markedly decreased in osteoporotic women [25] and ROS accumulation in the bone marrow is associated with bone loss in estrogen-deficient mice [26].

Epidemiological studies have found an association between dietary intake of vitamins C and E and bone mass / risk of hip fractures [27-29], and the administration of antioxidants, such as vitamins C and E and N-acetylcysteine, showed beneficial effects in individuals with osteoporosis [30-33]. Also ALA could have a therapeutic role in reducing bone loss associated with increased oxidative stress [34], although its clinical effect has not been determined.

Therefore, bone loss may also be hypothetically reduced by administration of antioxidant agents and the aim of this prospective comparative study was to evaluate the effect of oral supplementation of antioxidant agents containing ALA and powerful biological thiol antioxidant, on bone mineral density (BMD) of osteopenic postmenopausal women.

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Materials and Methods

A group of consecutive osteopenic postmenopausal women (n = 50) was enrolled in this prospective study. Women were randomly divided into two groups: ALA group (n = 25) received, for twelve months, oral tablets containing ALA (300 mg), vitamin C (30 mg), vitamin E (5 mg) and selenium (2.75 mg), twice daily, plus oral tablets containing calcium (500 mg) and vitamin D3 (400 IU), twice daily; the control group (n = 25) only received oral calcium (500 mg) and vitamin D3 (400 IU) with the same posology and for the same period.

The patients were selected according to the following inclusion criteria: age ≥ 45 years; clinical and hormonal diagnosis of postmenopause (serum estradiol levels < 110 pmol/l, serum follicle-stimulating hormone (FSH) levels > 30 IU/l); osteopenia (-2.5 < T-score < –1). The exclusion criteria were: early menopause (< 45 years); body mass index (BMI) ≥ 30; bone disorders except osteopenia; use of hormone replacement therapy (HRT) or other bone-active agents less than six months before enrolment.

The authors evaluated the T-score in the enrolment phase and the estimated BMD at baseline and after 12 months of treatment, by means of heel quantitative ultrasonometry (QUS). BMD estimate was performed with a bone sonometer applied to the non-dominant foot. Ultrasound frequency of 0.6 MHz was used to measure estimate BMD as g/cm^2 (CVs 3% for BMD, absolute precision 0.014 g/cm^2).

All values are presented as the mean value with range or standard deviation (SD). The statistical analysis was used by unpaired Student t-test. The level of statistical significance was set at $p < 0.05$.

Results

The study period of twelve months was completed by 44 patients out of 50 enrolled (23 in the ALA group and 21 in the control group). The women completing the study had a mean age of 60.1 years (range 49 - 75) and a BMI of 27.7 kg/m^2 (range 21.7 - 29.9); the menopause mean age was 50.7 years (range 45 - 56) and the mean duration of menopause was 9.5 years (range 1 - 27 years).

By group, the mean age in the ALA group was 60.7 ± 7.3 years and the BMI 27.3 ± 2.2 kg/m^2; in the control group, the mean age was 59.5 ± 6.5 years and the BMI 28.1 ± 1.5 kg/m^2. Concerning bone assessment, the baseline estimated BMD was 0.399 ± 0.028 g/cm^2 and 0.391 ± 0.022 g/cm^2 in the ALA and control group, respectively. The complete clinical baseline characteristics of the study population by intervention group are detailed in Table 1 and there were no significant differences ($p > 0.05$).

The treatment with ALA-containing antioxidant agents led to a significant better estimated BMD compared to the control group only receiving calcium and vitamin D3 (0.401 ± 0.026 vs 0.388 ± 0.025 g/cm^2). Nevertheless, this difference only reached the statistical significance ($p = 0.048$) (Figure 1).

Table 1. — *Baseline characteristics of the study population according to intervention group (mean ± S.D.).*

	ALA Group (n = 23)	Control Group (n = 21)	*p* value
Age (years)	60.7 ± 7.3	59.5 ± 6.5	0.297
BMI (kg/m^2)	27.3 ± 2.2	28.1 ± 1.5	0.107
Age of menopause (years)	51.1 ± 2.7	50.2 ± 3.0	0.151
Duration of menopause (years)	9.6 ± 7.0	9.5 ± 6.8	0.483
QUS Estimated BMD (g/cm^2)	0.399 ± 0.028	0.391 ± 0.022	0.142

ALA = alpha lipoic acid; BMD = bone mineral density; BMI = body mass index; QUS = quantitative ultrasonometry.

Discussion

The decrease in estrogen circulating levels during menopausal transition represents the main cause of bone loss [35]. A rapid decrease is, however, evident within the first five to ten years following menopause [36]. The physiological bone remodelling in this period is characterized by a relevant prevalence of resorption due to an increase of osteoclast pool size and activity, but the mechanisms through which estrogen deficiency stimulates bone resorption and impairs bone formation remain controversial and include direct effects of estrogen on osteoclasts and indirect effects that are cytokines-mediated. In addition, there is recent and increasing evidence that bone loss can also be mediated by oxidative stress [22-24].

Oxidative stress plays a pivotal role in many age-related degenerative processes, such as in case of postmenopausal bone loss by means of superoxide direct action and increase of osteoclastic differentiation [22-24]: estrogen deficiency lowers thiol antioxidant defenses in bone cells, thereby increasing ROS levels, which in turn induce expression of TNF, which causes bone loss [37]. A partial confirmation of these findings is the significant decrease of exogenous (vitamins A, C, and E) and endogenous (uric acid, superoxide dismutase, and glutathione peroxidase) antioxidizing agents in elderly osteoporotic women [25], and ROS accumulation in the bone marrow of ovariectomized mice with bone loss [26]. In particular, ovariectomy causes a substantial decrease in the levels of glutathione and thioredoxin that are the major tissue thiol antioxidants; which have been shown to prevent bone loss induced by estrogen deficiency [22-24].

ALA is a potent biological antioxidant and has been used to improve age-associated cardiovascular, cognitive, and neuromuscular deficits and has been implicated as a modulator of various inflammatory signaling pathways [7-9, 38-42]. More recently, the clinical role of ALA has been better defined as an inducer of cellular signaling pathways, insulin mimetic / hypotriglyceridemic agents, vasorelaxant / anti-hypertensive compounds, metal chelator and an adjuvant for neurocognitive function [17]: ALA may be effective in treating Alzheimer's disease and related dementias [43]; intravenous infusion reduces symptoms of diabetic peripheral neuropathy [44-46]; dietary supplementation prevents hypertension, insulin resistance, and aorta superoxide production in a rat model of hypertension induced by chronic glucose feeding [47]; ALA has recently been shown to exert potent antiobesity effects by suppressing hypothalamic adenosine monophosphate-activated protein kinase activity [48].

Moreover, there are some *in vitro* evidences suggesting

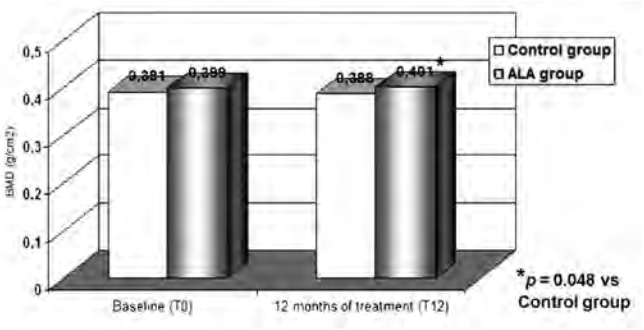

Figure 1. — Estimated BMD through heel QUS at baseline and mean after 12 months of treatment.
ALA = alpha lipoic acid; BMD = bone mineral density; QUS = quantitative ultrasonometry.

that ALA, like other thiol antioxidant agents, could have a therapeutic role in halting or reducing bone loss associated with increased oxidative stress: ALA suppresses osteoclastogenesis by direct inhibition of the receptor activator of nuclear factor-kappaB ligand (RANKL) mediated signals [49]; ALA showed to prevent bone resorption induced by RANKL and TNF-alpha [50]; a pretreatment of human bone marrow stromal cells (hBMSCs) with ALA prevented the apoptosis induced by TNF-alpha and hydrogen peroxide [34]. However, in the typical Western diet, ALA is not sufficiently supplied by diet and de novo synthesis takes place in the heart, liver, and testis [51], hence the concentration of free ALA in the circulation is very low and supplementation is necessary to reach potential therapeutic levels [34].

The purpose of this comparative investigation was to evaluate *in vivo* the effect of a 12-month oral supplementation with ALA, plus other antioxidant agents such as vitamin C, vitamin E, and selenium, on the BMD of a subset of 50 osteopenic postmenopausal women. BMD was measured with an ultrasound method, heel QUS measured on the non-dominant foot [52-54], not considered as the gold standard in the diagnosis of postmenopausal osteoporosis, but able to estimate the bone density decrease and predict the risk of fractures with respect to the conventional dual energy X-ray absorptiometry technique [55-59].

The results in the 44 patients that completed this one-year study showed that the association of antioxidant agents containing ALA to calcium and vitamin D3 led to a better estimated BMD compared to the control group only receiving calcium and vitamin D3 (0.401 vs 0.388 g/cm^2), even if this difference barely achieved a statistical significance ($p = 0.048$) (Figure 1).

These findings are consistent with the generic evidence that estrogen deficiency causes a lowering of antioxidant defenses in bone and that, whereas antioxidants prevent estrogen-deficient bone loss, lowering of oxidant defenses causes osteopenia [22-25, 34, 37]. In particular, this study partly confirmed a small *in vivo* experience on Parkinson's disease patients taking levodopa, in which overall BMD changes by ALA therapy were similar to the control group, but significantly greater at the trochanter (+4.6%, $p = 0.022$) after adjustment for BMI [60]. Furthermore, this experience confirms the association between dietary intake of vitamins C and E and bone mass [27-29] and their beneficial effects in osteoporotic patients [30-33]. Finally, the selenium supplementation and these clinical results are consistent with the evidence of decrease of glutathione peroxidase (selenium-containing) in elderly osteoporotic women [25].

Nevertheless, it should be noted that an analysis, not including ALA, of women participating in the large study Women's Health Initiative (WHI) does not support an independent association between intake or serum concentrations of antioxidants (vitamins A, C, E, retinol, beta-carotene, and selenium) and BMD [61].

Conclusions

This comparative study, although limited by a small population, showed that the oral supplementation of osteopenic postmenopausal women with ALA plus other antioxidant agents (vitamin C, vitamin E, and selenium) had favorable effects by mitigating bone loss compared to the supplementation of calcium and vitamin D3 only.

Even if the molecular impact of ALA and other antioxidant agents on bone turnover is yet not fully clear, it is apparent that oral supplementation could be clinically effective against oxidative stress-induced bone loss. Because current evidences mainly originate from *in vitro* studies, in particular regarding ALA, further controlled trials are needed to determine the potential to prevent bone loss, including postmenopausal osteoporosis.

References

[1] Bitar M.S., Wahid S., Pilcher C.W., Al-Saleh E., Al-Mulla F.: "Alpha-lipoic acid mitigates insulin resistance in Goto-Kakizaki rats". *Horm. Metab. Res*., 2004, 36, 542.
[2] Haugaard N., Levin R.M.: "Regulation of the activity of choline acetyl transferase by lipoic acid". *Mol. Cell. Biochem.,* 2000, *213,* 61.
[3] Packer L., Witt E.H., Tritschler H.J.: "Alpha-lipoic acid as a biological antioxidant". *Free Radic. Biol. Med.,* 1995, *19,* 227.
[4] Nguyen T., Yang C.S., Pickett C.B.: "The pathways and molecular mechanisms regulating Nrf2 activation in response to chemical stress". *Free Radic. Biol. Med.,* 2004, *37,* 433.
[5] Chen X.L., Kunsch C.: "Induction of cytoprotective genes through Nrf2/antioxidant response element pathway: a new therapeutic approach for the treatment of inflammatory diseases". *Curr. Pharm. Des.,* 2004, *10*, 879.
[6] Huang X., Atwood C.S., Hartshorn M.A., Multhaup G., Goldstein L.E., Scarpa R.C. *et al*.: "The A beta peptide of Alzheimer's disease directly produces hydrogen peroxide through metal ion reduction". *Biochemistry,* 1999, *38,* 7609.
[7] Suh J.H., Shenvi S.V., Dixon B.M., Liu H., Jaiswal A.K., Liu R.M. *et al*.: "Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid". *Proc. Natl. Acad. Sci USA,* 2004, *101*, 3381.
[8] Suh J.H., Zhu B.Z., deSzoeke E., Frei B., Hagen T.M.: "Dihydrolipoic acid lowers the redox activity of transition metal ions but does not remove them from the active site of enzymes". *Redox Rep*., 2004, *9*, 57.
[9] Suh J.H., Moreau R., Heath S.H., Hagen T.M.: "Dietary supplementation with (R)-alpha-lipoic acid reverses the age-related accumulation of iron and depletion of antioxidants in the rat cerebral cortex". *Redox Rep*., 2005, *10,* 52.

[10] Fonte J., Miklossy J., Atwood C., Martins R.: "The severity of cortical Alzheimer's type changes is positively correlated with increased amyloid-beta levels: resolubilization of amyloid-beta with transition metal ion chelators". *J. Alzheimers Dis.,* 2001, *3,* 209.

[11] Quinn J.F., Bussiere J.R., Hammond R.S., Montine T.J., Henson E., Jones R.E. *et al*.: "Chronic dietary alpha-lipoic acid reduces deficits in hippocampal memory of aged Tg2576 mice". *Neurobiol. Aging,* 2007, *28*, 213.

[12] Griffin W.S., Sheng J.G., Roberts G.W., Mrak R.E.: "Interleukin-1 expression in different plaque types in Alzheimer's disease: significance in plaque evolution". *J. Neuropathol. Exp. Neurol*., 1995, *54,* 276.

[13] Lander H.M., Tauras J.M., Ogiste J.S., Hori O., Moss R.A., Schmidt A.M.: "Activation of the receptor for advanced glycation end products triggers a p21(ras)-dependent mitogen-activated protein kinase pathway regulated by oxidant stress". *J. Biol. Chem*., 1997, *272,* 17810.

[14] Pocernich C.B., Butterfield D.A.: "Acrolein inhibits NADH-linked mitochondrial enzyme activity: implications for Alzheimer's disease". *Neurotox. Res.,* 2003*, 5,* 515.

[15] Arivazhagan P., Ramanathan K., Panneerselvam C.: "Effect of DL-alpha-lipoic acid on glutathione metabolic enzymes in aged rats". *Exp. Gerontol.,* 2001, *37,* 81.

[16] Simonian N.A., Coyle J.T.: "Oxidative stress in neurodegenerative diseases". *Ann. Rev. Pharmacol. Toxicol.,* 1996, *36,* 83.

[17] Shay K.P., Moreau R.F., Smith E.J., Smith A.R., Hagen T.M.: "Alpha-lipoic acid as a dietary supplement: Molecular mechanisms and therapeutic potential". *Biochim. Biophys. Acta,* 2009, *1790,* 1149.

[18] Romero F.J., Ordonez I., Arduini A., Cadenas E.: "The reactivity of thiols and disulfides with different redox states of myoglobin. Redox and addition reactions and formation of thiyl radical intermediates". *J. Biol. Chem.,* 1992, *267*, 1680.

[19] Jacob S., Streeper R.S., Fogt D.L., Hokama J.Y., Tritschler H.J., Dietze G.J. *et al*.: "The antioxidant alpha-lipoic acid enhances insulin-stimulated glucose metabolism in insulin-resistant rat skeletal muscle". *Diabetes,* 1996, *45,* 1024.

[20] Fuchs J., Schofer H., Milbradt R., Freisleben H.J., Buhl R., Siems W. *et al*.: "Studies on lipoate effects on blood redox state in human immunodeficiency virus infected patients". *Arzneimittelforschung,* 1993, *43,* 1359.

[21] Packer L., Tritsschler H., Wessel K.: "Neuroprotection by the metabolic antioxidant alpha-lipoic acid". *Free Rad. Biol. Med.,* 1997, *22*, 359.

[22] Lean J.M., Davies J.T., Fuller K., Jagger C.J., Kirstein B., Partington G.A. *et al*.: "A crucial role for thiol antioxidants in estrogen-deficiency bone loss". *J. Clin. Invest*., 2003, *112,* 915.

[23] Lean J., Kirstein B., Urry Z., Chambers T., Fuller K.: "Thioredoxin-1 mediates osteoclast stimulation by reactive oxygen species". *Biochem. Biophys Res. Commun.,* 2004, *321*, 845.

[24] Lean J.M., Jagger C.J., Kirstein B., Fuller K., Chambers T.J.: "Hydrogen peroxide is essential for estrogen-deficiency bone loss and osteoclast formation". *Endocrinology* 2005, *146,* 728.

[25] Maggio D., Barabani M., Pierandrei M., Polidori M.C., Catani M., Mecocci P. *et al*.: "Marked decrease in plasma antioxidants in aged osteoporotic women: Results of a cross-sectional study". *J. Clin. Endocrinol. Metab*., 2003, *88,* 1523.

[26] Grassi F., Tell G., Robbie-Ryan M., Gao Y., Terauchi M., Yang X. *et al*.: "Oxidative stress causes bone loss in estrogen-deficient mice through enhanced bone marrow dendritic cell activation". *Proc. Natl. Acad. Sci. USA,* 2007, *104*, 15087.

[27] Ilich J.Z., Brownbill R.A., Tamborini L.: "Bone and nutrition in elderly women: Protein, energy, and calcium as main determinants of bone mineral density". *Eur. J. Clin. Nutr.,* 2003, *57*, 554.

[28] Macdonald H.M., New S.A., Golden M.H., Campbell M.K., Reid D.M.: "Nutritional associations with bone loss during the menopausal transition: Evidence of a beneficial effect of calcium, alcohol, and fruit and vegetable nutrients and of a detrimental effect of fatty acids". *Am. J. Clin. Nutr.,* 2004, *79*, 155.

[29] Melhus H., Michaelsson K., Holmberg L., Wolk A., Ljunghall S.: "Smoking, antioxidant vitamins, and the risk of hip fracture". *J. Bone Miner. Res*., 1999, *14*, 129.

[30] Weber P.: "The role of vitamins in the prevention of osteoporosis - a brief status report". *Int. J. Vitam. Nutr. Res.,* 1999, *69*, 194.

[31] Hall S.L., Greendale G.A.: "The relation of dietary vitamin C intake to bone mineral density: Results from the PEPI study". *Calcif Tissue Int.,* 1998, *63,* 183.

[32] Patrick L.: "Toxic metals and antioxidants: Part II. The role of antioxidants in arsenic and cadmium toxicity". *Altern. Med. Rev.,* 2003, *8,* 106.

[33] Kurokouchi K., Kambe F., Yasukawa K., Izumi R., Ishiguro N., Iwata H. *et al*.: "TNF-alpha increases expression of IL-6 and ICAM-1 genes through activation of NF-kappaB in osteoblast-like ROS17/2.8 cells". *J. Bone Miner. Res.,* 1998, *13*, 1290.

[34] Byun C.H., Koh J.M., Kim D.K., Park S.I., Lee K.U., Kim G.S.: "Alpha-lipoic acid inhibits TNF-alpha-induced apoptosis in human bone marrow stromal cells". *J. Bone Miner. Res.,* 2005, *20,* 1125.

[35] Luisetto G., Zangari M., Tizian L., Nardi A., Ramazzina E., Adami S. *et al*.: "Influence of aging and menopause in determining vertebral and distal forearm bone loss in adult healthy women". *Bone Miner.,* 1993, *22,* 9.

[36] Riggs B.L., Khosla S., Melton L.J. III: "A unitary model for involutional osteoporosis: estrogen deficiency causes both type I and type II osteoporosis in postmenopausal women and contributes to bone loss in aging men". *J. Bone Miner. Res.,* 1998, *13,* 763.

[37] Jagger C.J., Lean J.M., Davies J.T., Chambers T.J.: "Tumor necrosis factor-alpha mediates osteopenia caused by depletion of antioxidants". *Endocrinology,* 2005, *146*, 113.

[38] Smith A.R., Shenvi S.V., Widlansky M., Suh J.H., Hagen T.M.: "Lipoic acid as a potential therapy for chronic diseases associated with oxidative stress". *Curr. Med. Chem.,* 2004, *11,* 1135.

[39] Liu J., Head E., Gharib A.M., Yuan W., Ingersoll R.T., Hagen T.M. *et al*.: "Memory loss in old rats is associated with brain mitochondrial decay and RNA/DNA oxidation: partial reversal by feeding acetyl-L-carnitine and/or (R)-alpha-lipoic acid". *Proc. Natl. Acad. Sci USA,* 2002, *99*, 2356.

[40] Lodge J.K., Traber M.G., Packer L.: "Thiol chelation of Cu2+ by dihydrolipoic acid prevents human low density lipoprotein peroxidation". *Free Radic. Biol. Med.,* 1998, *25*, 287.

[41] Anuradha B., Varalakshmi P.: "Protective role of DL-alpha-lipoic acid against mercury-induced neural lipid peroxidation". *Pharmacol. Res*., 1999, *39*, 67.

[42] Han D., Sen C.K., Roy S., Kobayashi M.S., Tritschler H.J., Packer L.: "Protection against glutamate-induced cytotoxicity in C6 glial cells by thiol antioxidants". *Am. J. Physiol.,* 1997, *273,* R1771.

[43] Holmquist L., Stuchbury G., Berbaum K., Muscat S., Young S., Hager K. *et al*.: "Lipoic acid as a novel treatment for Alzheimer's disease and related dementias". *Pharmacol. Ther*., 2007, *113*, 154.

[44] Reljanovic M., Reichel G., Rett K., Lobisch M., Schuette K., Moller W. *et al*.: "Treatment of diabetic polyneuropathy with the antioxidant thioctic acid (alpha-lipoic acid): a two year multicenter randomized double-blind placebo-controlled trial (ALADIN II). Alpha Lipoic Acid in Diabetic Neuropathy". *Free Radic. Res.,* 1999, *31*, 171.

[45] Ziegler D., Nowak H., Kempler P., Vargha P., Low P.A.: "Treatment of symptomatic diabetic polyneuropathy with the antioxidant alpha-lipoic acid: a meta-analysis". *Diabet. Med.,* 2004, *21,* 114.

[46] Ziegler D., Ametov A., Barinov A., Dyck P.J., Gurieva I., Low P.A. *et al*.: "Oral treatment with alpha-lipoic acid improves symptomatic diabetic polyneuropathy: the SYDNEY 2 trial". *Diabetes Care,* 2006*, 29,* 2365.

[47] Midaoui A., de Champlain J.: "Prevention of hypertension, insulin resistance, and oxidative stress by alpha-lipoic acid". *Hypertension,* 2002, *39*, 303.

[48] Kim M.S., Park J.Y., Namkoong C., Jang P.G., Ryu J.W., Song H.S. *et al*.: "Anti-obesity effects of alpha-lipoic acid mediated by suppression of hypothalamic AMP-activated protein kinase". *Nat. Med*., 2004, *10,* 727.

[49] Koh J.M., Lee Y.S., Byun C.H., Chang E.J., Kim H., Kim Y.H. *et al*.: "Alpha-lipoic acid suppresses osteoclastogenesis despite increasing the receptor activator of nuclear factor kappaB ligand/osteoprotegerin ratio in human bone marrow stromal cells". *J. Endocrinol.,* 2005, *185,* 401.

[50] Kim H.J., Chang E.J., Kim H.M., Lee S.B., Kim H.D., Su Kim G., *et al*.: "Antioxidant alpha-lipoic acid inhibits osteoclast differentiation by reducing nuclear factor-kappaB DNA binding and prevents in vivo bone resorption induced by receptor activator of nuclear factor-kappaB ligand and tumor necrosis factor-alpha". *Free Radicol. Biol. Med*., 2006, *40*, 1483.
[51] Morikawa T., Yasuno R., Wada H.: "Do mammalian cells synthesize lipoic acid? Identification of a mouse cDNA encoding a lipoic acid synthase located in mitochondria". *FEBS Lett*., 2001, *498,* 16.
[52] Langton C.M., Langton D.K.: "Male and female reference data for ultrasound measurement of the calcaneus within the UK adult population". *Br. J. Radiol*., 1997, *70*, 580.
[53] Ingle B.M., Sherwood K.E., Eastell R.: "Comparison of two methods for measuring ultrasound properties of the heel in postmenopausal women". *Osteoporos. Int*., 2001, *12,* 500.
[54] Louis O., Kaufman L., Osteaux M.: "Quantitative ultrasound of the calcaneus with parametric imaging: correlation with bone mineral density at different sites and with anthropometric data in menopausal women". *Eur. J. Radiol*., 2000, *35,* 65.
[55] Von Stetten E., Ouellet H., Wilson K., Steiger P., Stein J.A.: "European caucasian female reference values for the Sahara clinical bone sonometer". Bedford (USA), Hologic Inc., 1998.
[56] Ponteggia L., Di Cato M., Ponteggia F., Pica M., Puxeddu A., Coaccioli S.: "Evaluation of the peak bone mass by quantitative heel ultrasound in young women of the centre of Italy". *Reumatismo*, 2003, *55*, 34.
[57] He Y.Q., Fan B., Hans D., Li J., Wu C.Y., Njeh C.F. *et al*.: "Assessment of a new quantitative ultrasound calcaneus measurement: precision and discrimination of hip fractures in elderly women compared with dual X-ray absorptiometry". *Osteoporos. Int*., 2000, *11,* 354.
[58] Adami S., Bertoldo F., Brandi M.L., Cepollaro C., Filipponi P., Fiore E. *et al*.: "Guidelines for the diagnosis, prevention and treatment of osteoporosis". *Reumatismo*, 2009, *61*, 260.
[59] Cummings S.R., Bates D., Black D.M.: "Clinical use of bone densitometry: scientific review". *JAMA,* 2002, *288*, 1889.
[60] Lee S.H., Kim M.J., Kim B.J., Kim S.R., Chun S., Ryu J.S. *et al*.: "Homocysteine-lowering therapy or antioxidant therapy for bone loss in Parkinson's disease". *Mov. Disord*., 2010, *25,* 332.
[61] Wolf R.L., Cauley J.A., Pettinger M., Jackson R., Lacroix A., Leboff M.S. *et al*.: "Lack of a relation between vitamin and mineral antioxidants and bone mineral density: results from the Women's Health Initiative". *Am. J. Clin. Nutr*., 2005, *82,* 581.

Address reprint requests to:
G. MAININI, M.D.
Via Diaz, 77
80055 Portici, Naples (Italy)
e-mail: giampaolomainini@libero.it