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Effects of nano calcium carbonate and nano calcium citrate on toxicity in ICR mice and on bone mineral density in an ovariectomized mice model

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Abstract

Taking calcium supplements can reduce the risk of developing osteoporosis, but they are not readily absorbed in the gastrointestinal tract. Nanotechnology is expected to resolve this problem. In the present study, we examined whether the bioavailability of calcium carbonate and calcium citrate can be improved by reducing the particle size. The morphology of nano calcium carbonate and nano calcium citrate was characterized by dynamic laser-light scattering (DLS), field-emission scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM). The measurements obtained from DLS, FE-SEM and TEM were comparable. Acute and sub-chronic toxicity tests were performed to establish the safety of these products after oral administration. The no-observed-adverse-effect levels of nano calcium carbonate and nano calcium citrate were 1.3 and 2.3 g kg^{-1} body weight, respectively. The results of our *in vivo* studies indicate that administering nano calcium carbonate and nano calcium citrate can enhance the serum calcium concentration and maintain the whole-body bone mineral density in ovariectomized mice. These data suggest that nano calcium carbonate and nano calcium citrate are more bioavailable than micro calcium carbonate and micro calcium citrate, respectively.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Osteoporosis is a silent disease that causes bones to become more porous, and is usually symptomless until severe backache or hip fracture occurs, particularly in the elderly. Hormone deficiency in the elderly can result in a negative whole-body calcium balance, leading to higher rates of bone loss. Bone mass is maintained at a constant level by the balance between osteoclastic bone resorption and osteoblastic bone formation (Erlebacher *et al* 1998, Oursler *et al* 1991, Hock *et al* 2001, Britto *et al* 1994). Numerous data indicate that calcium inhibits the formation and activity of osteoclasts and stimulates the activity of osteoblasts. In addition, high ionic calcium has been shown to promote osteoclast apoptosis (Sugimoto *et al* 1993, 1994). Calcium is a fundamental component of hydroxyapatite, which is one of the minerals involved in the construction of bone, and is required for the normal growth, development and maintenance of the skeleton. Sufficient dietary calcium is necessary for bone health during growth as well as in the elderly (Rizzoli 2008, Bonjour *et al* 2001). Supplementation studies of postmenopausal women have revealed an increase in bone loss related to reduced calcium intake (Farrin *et al* 2008, Mavroeidi *et al* 2009, Swaim *et al* 2008, Dawson-Hughes *et al* 1997, Klibanski *et al* 2001, Reid *et al* 1993). However, supplementation with calcium particles that are too large and too hard, such as commercially

available micro calcium, results in low absorption efficiency. The dosage of calcium therefore needs to be raised to enable the optimal amount for absorption; however, a high dosage is not advantageous to the elderly and is not environmentally friendly. Nanotechnology is expected to resolve this issue.

Nanotechnology was applied in 2008 to health food science to stabilize bioactive materials and improve their bioavailability (Mozafari *et al* 2008). Kim *et al* showed that propolis nanofood, unlike free propolis, is readily dispersed in aqueous media, and its therapeutic efficacy *in vitro* against a panel of human pancreatic cancer cell lines is comparable to that of free propolis (Kim *et al* 2008). Hatanaka *et al* revealed that nanosize coenzyme Q10 exhibits high solubility and dispersibility in water. Although similar kinetic values were seen for nano coenzyme Q10 and crystalline coenzyme Q10, the area under the curve was 1.7 times higher for the former than for the latter (Hatanaka *et al* 2008). Greenhalgh *et al* showed that nanoparticle emulsions are highly effective in the treatment of dermal and systemic methicillin-resistant *Staphylococcus aureus* infections in mouse models (Greenhalgh and Turos 2009). Park *et al* found that consumption of nano-calcium-enriched milk resulted in an increase in the urinary excretion of calcium and a decrease in that of deoxypyridinoline and hydroxyproline in ovariectomized (OVX) rats (Park *et al* 2008). There is still little information available about the effects of nano calcium carbonate and nano calcium citrate supplements on bone mineral density (BMD). This study characterized the size distribution and morphology of nano calcium carbonate and nano calcium citrate. Because nanoscale supplements are novel formulas in health foods, we have to determine the acute toxicity, sub-chronic toxicity and bioavailability of both sexes of mice in advance. Moreover, the anti-osteoporosis activity was demonstrated by an OVX mice model. A bilateral OVX ICR mouse model was utilized to mimic the condition in postmenopausal women. BMD was examined after administering nano calcium carbonate and nano calcium citrate, respectively.

2. Materials and methods

2.1. Preparation of nano calcium carbonate and nano calcium citrate

Micro calcium carbonate was provided by Diamond Nano-Biochem (Taichung, Taiwan, ROC), and micro calcium citrate was purchased from TOPO Biotechnology (Taipei, Taiwan, ROC). Calcium carbonate and calcium citrate were nanoscaled with the aid of a pulsed air-flow pulverizer (Diamond Nano-Biochem). The powders were stored at room temperature until analysis.

2.2. Measurement of the particle hydrodynamic diameters

The hydrodynamic diameters of nano calcium carbonate and nano calcium citrate were examined by dynamic laser-light scattering (DLS) in the single-scattering regime with $\lambda = 532$ nm (Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK). Typically, particles were suspended in Milli-Q water (1.5 ml) at a concentration of 0.1 mg ml^{-1} and sonicated for 30 s. The suspension was put into a cuvette at 25 ± 1 °C to enable particle size analysis. The viscosity and refractive index of the continuous phase were set to those specific to water.

2.3. Field-emission scanning electron microscopy

To demonstrate the morphology of the particles, the samples were examined by field-emission scanning electron microscopy (FE-SEM) using a Hitachi S-4100 instrument (Hitachi Instruments, Tokyo, Japan) operating at 15 kV. The FE-SEM samples were prepared by suspending 1 mg of nanoparticles in 1 ml of Milli-Q water; 200 μl of the suspended nanoparticles was then pipetted onto a holder and dehydrated. The electrical conductivity of the samples was enhanced by coating them with platinum via sputtering (SPI-Module Sputter Coater, Structure Pro, PA, USA) before examination.

2.4. Transmission electron microscopy

High resolution images were obtained by transmission electron microscopy (TEM) using a JEM-2010 instrument (JEOL, Tokyo, Japan) at an accelerating voltage of 200 kV. A dilute suspension (1 mg ml^{-1}) of particles was prepared in Milli-Q water and sonicated for 30 s. The aqueous dispersion was thereafter deposited on a 200-mesh carbon-coated copper grid and dried at room temperature overnight before examination.

2.5. Animals

Laboratory-bred, eight- to ten-week-old ICR mice (National Taiwan University Hospital, Taipei, Taiwan, ROC) of both sexes were used in the toxicity study and only females were used in the biofunctional study. The animals were housed at 21–25 °C, a relative humidity of 30–70% and a 12 h/12 h day/night cycle. Pelleted mouse feed (MF Laboratory Animal Diet, Oriental Yeast, Tokyo, Japan) and reverse-osmosis water were provided *ad libitum*. All procedures used in the study were approved by the Institutional Animal Ethics Committee. Bilateral ovariectomy was performed from a dorsal approach at six- to eight-week-old mice. Surgical removal of the ovaries is a well-represented approach to mimic the postmenopausal condition in mice. Briefly, mice were anesthetized with pentobarbital sodium (60 mg kg^{-1} body weight). Hair was cut over the surgical area and scrubbed with iodine. A small incision (∼1.0 cm) was made in the skin between the middle of the back, starting at the last rib. The skin was moved to one lateral side, and a small incision was made through the peritoneal lining. The entire ovary was removed with a single cut between the uterine horn and oviduct on each side. The skin was then recovered with a surgical staple.

2.6. The seven-day acute oral toxicity test

Standard acute toxicological evaluations of the ICR mice were performed in the initial assessment of the effects of nanoscale calcium carbonate internalization. Following overnight fasting, ICR mice were weighed and randomly divided into five groups of 16 animals (eight females and eight males). Placebo (deionized water), vitamin D_3 (26I U/kg body weight) plus

micro calcium carbonate (1.3 g kg^{-1} body weight), vitamin D_3 plus nano calcium carbonate (1.3 g kg^{-1} body weight), vitamin D_3 plus micro calcium citrate (2.3 g kg^{-1} body weight) or vitamin D_3 plus nano calcium citrate (2.3 g kg^{-1} body weight) was administered in a single dose by gavage using a gastric intubation tube. According to molecular weight, we kept the same quantity of calcium. On day seven, all the animals were weighed and any signs of toxicity were noted.

2.7. Repeated-dose 28-day sub-chronic oral toxicity

Seventy-eight female mice were randomly divided into 13 groups of 16 animals (eight females and eight males). Vitamin D_3 (26I U/kg body weight) plus micro calcium carbonate or nano calcium carbonate was administered by gavage to groups of mice at doses of 1.3, 0.13 or 0.013 g kg^{-1} body weight. Similarly, vitamin D_3 plus micro calcium citrate or nano calcium citrate was administered by gavage to groups of mice at doses of 2.3, 0.23 or 0.023 g kg^{-1} body weight; the control group received the vehicle only. All experimental animals were observed every day for general signs and symptoms of toxicity. The body weights were recorded prior to the commencement of the study. All of the animals were weighed on day 28, and then sacrificed after an overnight fast.

2.8. In vivo evaluation of serum calcium

Ninety-six female mice were randomly divided into four groups of 24 animals ($n = 6$ in each group). Vitamin D_3 (26I U/kg body weight) plus micro calcium carbonate or nano calcium carbonate was administered by gavage to groups of mice at a dose of 1.3 g kg^{-1} body weight. Similarly, vitamin D_3 plus micro calcium citrate or nano calcium citrate was administered by gavage to groups of mice at a dose of 2.3 g kg^{-1} body weight. At various time intervals (2, 6, 12 and 24 h) blood samples were collected from the inferior vena cava of each mouse before autopsy. Serum calcium ion concentration was estimated using analysis kits (HUMAN, Wiesbaden, Germany).

2.9. Biofunctionality of nano calcium carbonate and nano calcium citrate on OVX ICR mice

All operative procedures were performed under general anesthesia (intra-peritoneal injection of pentobarbital sodium, at a dose of 60 mg kg^{-1} body weight) and using aseptic surgical techniques. Sham surgery ($n = 6$; SHAM) or bilateral ovariectomy ($n = 30$; OVX) was performed on 6- to 8-week-old mice. In the sham operation, ovaries were exteriorized and then replaced. At two months post-surgery, the OVX group was further randomly divided into five groups of six animals. Placebo (deionized water), vitamin D_3 (26I U/kg body weight) plus micro calcium carbonate (1.3 g kg^{-1} body weight), vitamin D_3 plus nano calcium carbonate (1.3 g kg^{-1} body weight), vitamin D_3 plus micro calcium citrate (2.3 g kg^{-1} body weight) or vitamin D_3 plus nano calcium citrate (2.3 g kg^{-1} body weight) was administered every day by gavage to the five groups of mice for 28 days. All of the animals were killed by an overdose of anesthetic on day 28. The BMD of the whole body was analyzed by dual-energy x-ray absorptiometry (pDEXA, Norland Stratec, Fort Atkinson, WI, USA).

Table 1. Hydrodynamic diameter of particles, as determined by dynamic laser-light scattering (DLS). Data are mean ± SE values.

Particle	Size from volume distribution (nm)
Micro calcium carbonate	3773 ± 759
Nano calcium carbonate	151 ± 19
Micro calcium citrate	1793 ± 382
Nano calcium citrate	398 ± 41

2.10. Statistical analysis

Data are expressed as mean ± SE values and were analyzed statistically using one-way ANOVA followed by the least-significant-difference multiple comparison test using SPSS version 14.0 to establish the significance of any differences. The level of statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. Characterization of the particles

DLS was used to quantitatively measure the size and size distribution of the particles. The diameters of the micro and nano calcium carbonate particles were 3773 ± 759 nm and 151±19 nm, respectively. The diameters of the micro and nano calcium citrate particles were 1793±382 nm and 398±41 nm, respectively. The findings are listed in table 1. FE-SEM was utilized to examine the morphology, size and shape of the particles (see figure 1). The obtained images revealed the smooth surface morphology of nano calcium carbonate and nano calcium citrate. The diameter of the nano calcium carbonate particles ranged from 100 to 200 nm, and that of the nano calcium citrate particles ranged from 200 to 400 nm. The nano calcium carbonate and nano calcium citrate particles were spherical. Nano calcium carbonate and nano calcium citrate aggregates were also seen on the FE-SEM images.

Figures 2(a) and (b) show TEM micrographs of nano calcium carbonate and nano calcium citrate, respectively, and confirm that the particles have a spherical-to-oval morphology. The diameter of the nano calcium carbonate particles ranged from 100 to 200 nm and that of nano calcium citrate ranged from 200 to 400 nm. The measurements made using DLS, FE-SEM and TEM were comparable. These findings provide a direct measure of particle size and size distribution in solution.

We were able to produce uniform nano calcium carbonate and nano calcium citrate particles with the aid of a pulsed air-flow pulverizer. According to the National Nanotechnology Initiative (2006), 'Nanotechnology is the understanding and control of matter at dimensions of roughly 1100 nm'. However, this definition of nanoparticle size was established for chemistry, physics and electronics; in the fields of nanofood and nano herb research, the particle size of interest is 1–1000 nm. Preetz *et al* developed stable polyelectrolyte nanocapsules with an average size of 130 nm. These nanocapsules could be extremely useful to the food

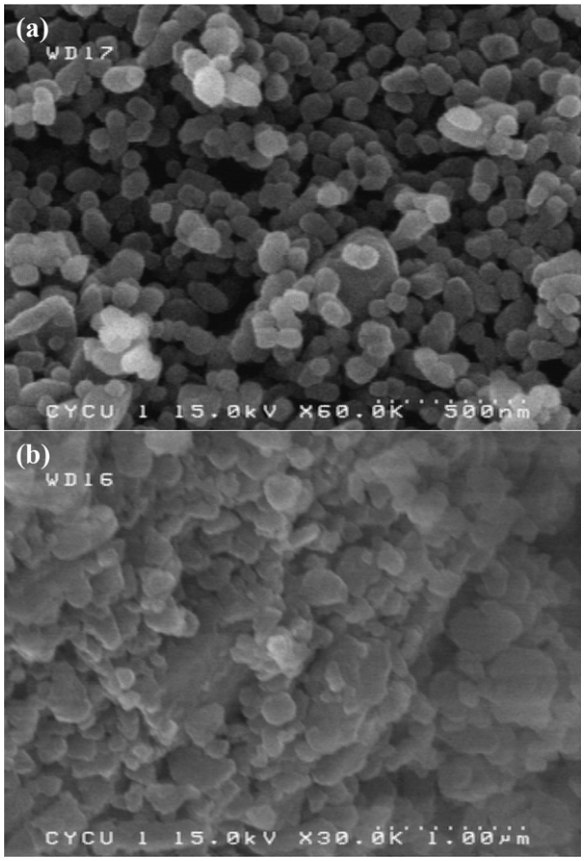

Figure 1. Field-emission scanning electron microscopy photomicrographs of particles on a solid substrate: (a) nano calcium carbonate and (b) nano calcium citrate.

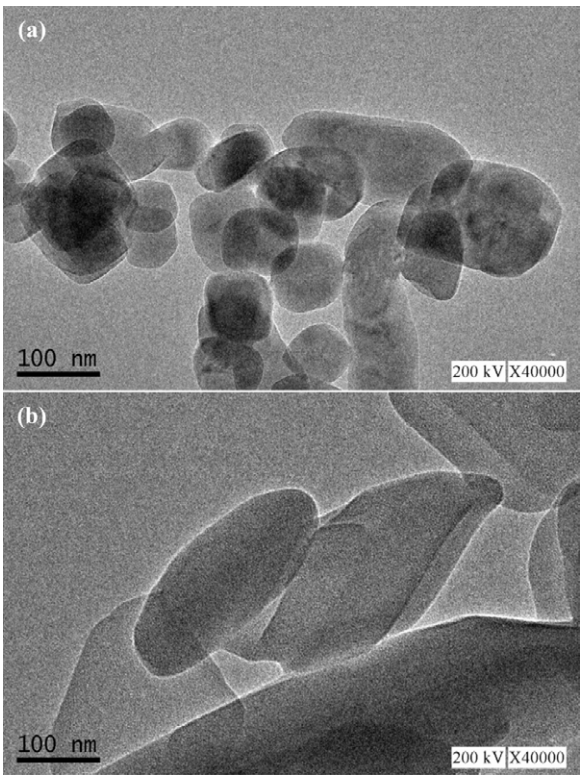

Figure 2. Transmission electron microscopy images of nanoparticles: (a) nano calcium carbonate and (b) nano calcium citrate.

and pharmaceutical industries for incorporating lipophilic substances (Preetz *et al* 2008). Semo *et al* demonstrated that reassembled casein micelles with a size of 100–200 nm can be used for the nano-encapsulation of hydrophobic nutraceutical substances for potential enrichment of low- or non-fat food products (Semo *et al* 2007). Nahar *et al* indicated that gelatin nanoparticles with a size of 213 ± 10 nm could optimize amphotericin B delivery in terms of both cost and safety (Nahar *et al* 2008).

3.2. The seven-day acute oral toxicity test

Table 2 indicates that the no-observed-adverse-effect level (NOAEL) of both micro calcium carbonate and nano calcium carbonate was 1.3 g kg^{-1} body weight. Similarly, the NOAEL of both micro calcium citrate and nano calcium citrate was 2.3 g kg^{-1} body weight. Throughout the study, no unusual behavior or differences between groups were observed, including labored breathing, difficulties moving, hunching or unusual interactions with cage mates.

3.3. Repeated-dose 28-day sub-chronic oral toxicity

The 28-day sub-chronic toxicity test was conducted in mice with micro and nano calcium carbonate at doses of 1.3, 0.13 and 0.013 g kg^{-1} body weight, and micro and nano calcium citrate at doses of 2.3, 0.23 and 0.023 g kg^{-1} body weight. Table 3 indicates that daily oral administration of micro calcium carbonate, nano calcium carbonate, micro calcium citrate or nano calcium citrate did not produce any obvious symptoms of toxicity or mortality, even at the highest dose administered. Overall, body weight increased slightly but insignificantly throughout the study in all groups as the mice matured. A careful and extensive necropsy revealed no gross organ changes. These results support the safety of micro and nano calcium carbonate and calcium citrate for oral consumption.

3.4. Serum calcium concentration

Figure 3 shows that serum calcium concentrations were significantly higher in the nano calcium carbonate group than in the micro calcium carbonate group at 6 h post-administration ($*p < 0.05$). Serum calcium concentrations tended to be higher in the nano calcium carbonate group than in the micro calcium carbonate group at 2 h post-administration. Similarly, serum calcium concentrations were significantly higher in the nano calcium citrate group than in the micro calcium citrate group at both 6 and 12 h post-administration (see figure 4; $*p < 0.05$, $**p < 0.01$). Serum calcium concentrations also tended to be higher in the nano calcium citrate group than in the micro calcium citrate group at both 2 and 24 h post-administration. The nano calcium carbonate and nano calcium citrate thus exhibited a higher efficacy than their microsized equivalents.

Table 2. Body weight and mortality during the seven-day acute toxicity test that assessed the acute toxicity of micro calcium carbonate (1.3 g kg^{-1} body weight), nano calcium carbonate (1.3 g kg^{-1} body weight), micro calcium citrate (2.3 g kg^{-1} body weight) or nano calcium citrate (2.3 g kg^{-1} body weight), which was administered orally to the mice. Data are mean ± SE values.

Dose	Sex (n)	Initial body weight (g)	Final body weight (g)	Mortality dead/treated
Control	Male (8)	33.2 ± 3.3	35.3 ± 3.9	0/8
(0 g kg^{-1} body weight)	Female (8)	32.5 ± 3.7	34.2 ± 3.8	0/8
Micro calcium carbonate	Male (8)	33.4 ± 3.2	34.9 ± 3.2	0/8
(1.3 g kg^{-1} body weight)	Female (8)	32.7 ± 3.4	34.3 ± 3.6	0/8
Nano calcium carbonate	Male (8)	32.5 ± 3.6	33.7 ± 4.1	0/8
(1.3 g kg^{-1} body weight)	Female (8)	33.1 ± 2.9	34.9 ± 3.8	0/8
Micro calcium citrate	Male (8)	33.5 ± 3.1	35.4 ± 4.0	0/8
(2.3 g kg^{-1} body weight)	Female (8)	32.4 ± 3.6	35.6 ± 3.6	0/8
Nano calcium citrate	Male (8)	34.2 ± 3.8	36.2 ± 3.4	0/8
(2.3 g kg^{-1} body weight)	Female (8)	33.6 ± 4.2	34.2 ± 3.4	0/8

Table 3. Body weight and mortality during the 28-day sub-chronic toxicity test. Data are mean ± SE values.

Dose	Sex (n)	Initial body weight (g)	Final body weight (g)	Mortality (%)
Control	Male (8)	31.2 ± 3.3	35.3 ± 3.9	0
	Female (8)	32.5 ± 3.6	36.1 ± 3.7	0
Micro calcium carbonate	Male (8)	33.4 ± 3.2	36.8 ± 3.5	0
(1.3 g kg^{-1} body weight)	Female (8)	32.1 ± 3.9	36.2 ± 3.6	0
Micro calcium carbonate	Male (8)	32.4 ± 3.2	36.3 ± 3.5	0
(0.13g/ body weight)	Female (8)	31.9 ± 3.1	35.2 ± 3.3	0
Micro calcium carbonate	Male (8)	33.4 ± 3.2	37.3 ± 3.7	0
(0.013 g kg^{-1} body weight)	Female (8)	33.1 ± 3.1	37.2 ± 3.3	0
Nano calcium carbonate	Male (8)	32.4 ± 3.6	36.4 ± 4.1	0
(1.3 g kg^{-1} body weight)	Female (8)	33.1 ± 2.9	36.9 ± 3.7	0
Nano calcium carbonate	Male (8)	32.4 ± 3.3	36.4 ± 3.9	0
(0.13 g kg^{-1} body weight)	Female (8)	33.8 ± 3.6	37.2 ± 3.4	0
Nano calcium carbonate	Male (8)	32.9 ± 3.3	36.4 ± 3.4	0
(0.013 g kg^{-1} body weight)	Female (8)	32.5 ± 3.2	36.2 ± 3.3	0
Micro calcium citrate	Male (8)	33.5 ± 3.1	37.3 ± 3.9	0
(2.3 g kg^{-1} body weight)	Female (8)	34.2 ± 3.8	37.7 ± 3.6	0
Micro calcium citrate	Male (8)	33.7 ± 3.1	37.3 ± 3.5	0
(0.23 g kg^{-1} body weight)	Female (8)	32.2 ± 3.2	37.1 ± 3.3	0
Micro calcium citrate	Male (8)	31.4 ± 3.1	35.3 ± 3.5	0
(0.023 g kg^{-1} body weight)	Female (8)	34.3 ± 3.2	38.1 ± 3.3	0
Nano calcium citrate	Male (8)	32.4 ± 3.2	35.9 ± 3.4	0
(2.3 g kg^{-1} body weight)	Female (8)	33.6 ± 4.2	36.1 ± 4.4	0
Nano calcium citrate	Male (8)	32.4 ± 3.8	36.4 ± 3.4	0
(0.23 g kg^{-1} body weight)	Female (8)	34.1 ± 3.2	37.1 ± 4.1	0
Nano calcium citrate	Male (8)	32.7 ± 3.7	37.4 ± 3.4	0
(0.023 g kg^{-1} body weight)	Female (8)	33.2 ± 3.2	37.1 ± 3.7	0

Intestinal calcium absorption occurs transcellularly in the duodenum and paracellularly throughout the small intestine (Bronner and Pansu 1999). The sojourn time of chyme in the mouse duodenum is a matter of minutes, whereas in the lower half of the small intestine it is well over 2 h, which is similar to that measured in people (Bronner and Pansu 1999, Bronner 2009). The primary site of calcium absorption is the small intestine, where some 90% of calcium is absorbed. These reports support the finding that serum calcium concentration was up-regulated following administration of nano calcium citrate and nano calcium carbonate, especially at 6 and 12 h post-administration.

3.5. BMD of the whole body

The BMD of the whole body in OVX mice was significantly lower than in SHAM mice (see figure 5; $p < 0.001$). The BMD value of the whole body in the micro and nano calcium carbonate and calcium citrate-treated OVX mice was greater than in the placebo-treated OVX mice ($p < 0.001$). The BMD of the nano calcium carbonate-treated OVX mice was significantly higher than in the micro calcium carbonate-treated OVX mice ($p < 0.001$). The BMD of the nano calcium citrate-treated OVX mice was significantly higher than in the micro calcium citrate-treated OVX mice ($p < 0.001$).

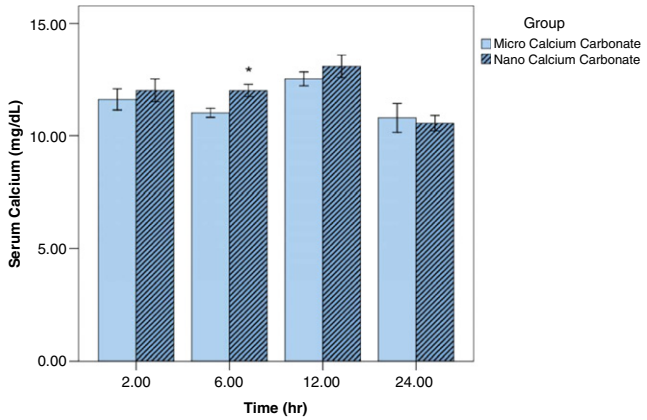

Figure 3. Serum calcium concentrations of micro calcium carbonate (1.3 g kg^{-1} body weight) and nano calcium carbonate (1.3 g kg^{-1} body weight) groups at 2, 6, 12 and 24 h post-administration. Data are mean ± SE values ($n = 6$ for all groups; * significant difference between micro calcium carbonate and nano calcium carbonate group, $p < 0.05$).

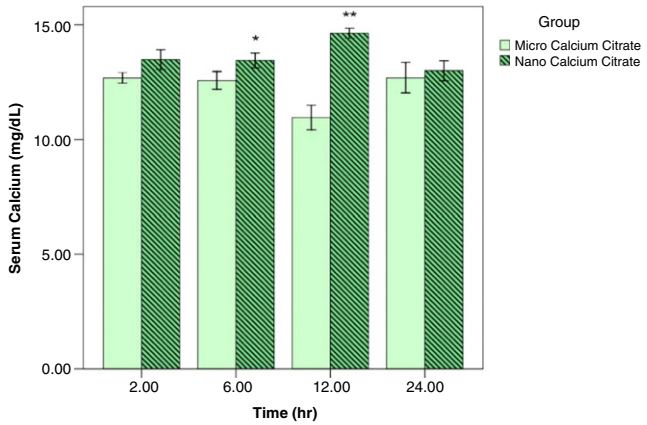

Figure 4. Serum calcium concentrations of micro calcium citrate (2.3 g kg^{-1} body weight) and nano calcium citrate (2.3 g kg^{-1} body weight) groups at 2, 6, 12 and 24 h post-administration. Data are mean ± SE values ($n = 6$ for all groups; * significant difference between micro calcium citrate and nano calcium citrate groups, $p < 0.05$).

In addition, the BMD of the nano calcium citrate-treated OVX mice was equal to that in the SHAM mice, and it was significantly higher than in the micro and nano calcium carbonate-treated OVX mice ($p < 0.001$). The BMD was maintained by nano calcium citrate treatment in OVX mice.

The most important finding of the present study relates to the significant estrogen deficiency observed in OVX mice (Usui *et al* 2004), rats (Fanti *et al* 1998, Finkelman *et al* 1992, Aitken *et al* 1972) and postmenopausal women (Richelson *et al* 1984, Dequeker and Geusens 1985, Riggs *et al* 1998). Estrogen enhances active calcium absorption (Gallagher *et al* 1980). Therefore, the BMD of OVX mice was significantly attenuated compared to the SHAM mice.

The benefits of calcium intervention appear to be more marked in late postmenopausal life than at the perimenopause

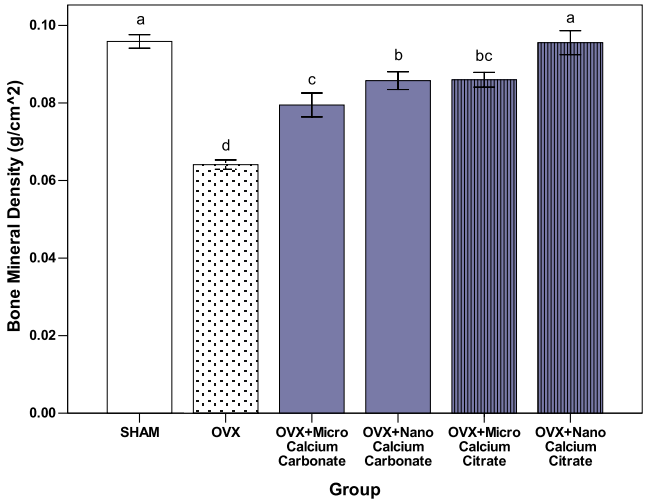

Figure 5. Bone mineral density (g cm^{-2}) of sham-operated (SHAM) mice, ovariectomized (OVX) mice and OVX mice treated with 1.3 g kg^{-1} body weight micro calcium carbonate, 1.3 g kg^{-1} body weight nano calcium carbonate, 2.3 g kg^{-1} body weight micro calcium citrate or 2.3 g kg^{-1} body weight nano calcium citrate for 28 days. Data are mean ± SE values ($n = 6$ for all groups; the same letters indicate no significant differences, $p < 0.001$).

(Dawson-Hughes *et al* 1990). Prince *et al* also indicated that calcium supplementation could enhance the uptake of calcium and maintain BMD in postmenopausal women (Prince *et al* 1995). For that reason, calcium supplementation with fine bioavailability could protect OVX mice from osteoporosis. In the study presented here, administration of nano calcium carbonate and nano calcium citrate to OVX mice was more effective at inducing calcium uptake (as shown by increased serum calcium concentrations), and maintained their BMD.

4. Conclusion

The field of health food and medicine could be revolutionized by nanotechnology. Although further research is required in this area, our results related to the problem of the poor bioavailability of current available calcium supplements have demonstrated the essential non-toxicity *in vivo* and the efficacy with regard to serum calcium concentrations and BMD in OVX mice of nano calcium carbonate and nano calcium citrate. Nano calcium carbonate and nano calcium citrate are thus potential and convenient calcium supplements.

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