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Effect of nanometer pearl powder on calcium absorption and utilization in rats

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Abstract

The calcium absorption and utilization in rats fed nanometer pearl powders diets was evaluated. The bone and serum calcium content, femur weight and length of rats fed the pearl powders diets was higher ($P < 0.05$) than those of rats fed the basic laboratory chow diet with low content of calcium. These parameters were significantly lower in rats fed nanometer pearl powders diets compared with those in rats fed micrometer pearl powders diets ($P < 0.05$). The results indicate that nanometer pearl powders diets may significantly increase calcium bioavailability and have important nutritional benefits based on the evaluation in the rats growth and development model.

Keywords: Nanometer pearl powder; Micrometer pearl powder; Calcium absorption; Calcium utilization

1. Introduction

The nacre (mother of pearl) layer of the giant oyster (Pinctada maxima) shell can initiate bone formation in vitro (Lopez et al., 1992) and in vivo (Atlan, Balmain, Berland, Vidal, & Lopez, 1997). Nacre is biocompatible and has osteogenic properties (Delattre, Catonne, Berland, Borzeix, & Lopez, 1997). Nacre implants in sheep (Delattre et al., 1997), rats (Atlan et al., 1999) and human for alveolar bone defects (Atlan et al., 1997) caused differentiation of osteoblasts leading to the formation of mature, functional bone (Sriamornsak, Sungthongjeen, & Puttipipatkhachorn, 2007). Nacre also initiates mineralization by human osteoblasts in vitro (Liao, Brandsten, Lundmark, & Li, 1997). Lopez et al. (1992) have suggested that nacre acts on osteogenesis via its organic matrix, which contains diffusible, water-soluble factors (Westbroek & Marin, 1998).

A natural pearl is formed by deposits of nacre around an irritant which accidentally lodges within the body of an oyster (Huang, Yu, & Xiao, 2006; Silve et al., 1992). A number of studies have indicated that pearl is an excellent source of calcium that is beneficial to the body. It contains over 25 organic salts and 10 amino acids, providing a rich source of organic calcium, selenium, zinc, and other trace metal elements. As the generation of people over 60 grows, there has been an enormous increase in the amount of osteoporosis, joint pain, and arthritis and a general decline in the quality of health (Atlan et al., 1997). Studies show that many falls experienced by the elderly are actually caused by a hip or knee spontaneously fracturing just before the fall occurs (Sriamornsak et al., 2007). Nanometer pearl powder (average diameter of 40–80 nm) is derived by thoroughly grinding natural pearls through a patented cutting-edge technology that makes the ingredients more bioavailable. Because the nanometer pearl powder is easily absorbed

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into the bloodstream, it replenishes the calcium deficit within a short period of time, as well as supplies some trace metal elements and thereby improve the immune system.

This study reports investigations on the effects of nanometer pearl powder on calcium absorption and utilization in rats with the aim to provide a scientific theory for future pharmacological application of nanometer pearl powder.

2. Materials and methods

2.1. Materials

Nanometer and micrometer pearl powders were kindly provided by the ZheJiang ChangShengNiao Medicine Co., Ltd. (Hangzhou, China). The calcium contents (35.0% and 34.6%) in both pearl powder loads were just taken from the chemical analysis datasheet from the provider. Other chemicals and reagents were analytical grade.

2.2. Animal and diets

One hundred ten, 21 days, SD rats weighing about 70 g, were obtained from the Laboratory Animal Center of Second Military Medical University (Shanghai, China). Animals were kept in an environmentally controlled breeding room (temperature: 20 ± 2 °C, humidity: $60 \pm 5\%$, 12 h dark/light cycle). They were fed basic laboratory chow diet with low level of calcium (0.1%) and allowed to free access to tap water for 4 days before the experiments. Experiments were conducted in accordance with the declaration of Helsinki and/or with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the China National Institutes of Health. One kilogram basic laboratory chow diet contained 320 g corn, 300 g wheat, 150 g soybean meal, 200 g vegetable protein powder, 10 g salt and 20 g soybean salad oil.

$$\text{Retention rate of calcium in femurs } (\%) = \frac{\text{calcium content in femurs of experimental rats} - \text{one in femurs of control rats}}{\text{total calcium intake}} \times 100$$

$$\text{Total calcium intake} = \text{total feedstuff} \times \text{calcium content in feedstuff}$$

$$\text{Absorption rate of calcium } (\%) = \frac{\text{total calcium intake} - \text{calcium content in feces}}{\text{total calcium intake}} \times 100$$

$$\text{Retention rate of calcium } (\%) = \frac{\text{total calcium intake} - \text{calcium content in feces} - \text{calcium content in urine}}{\text{total calcium intake} - \text{calcium content in feces}} \times 100$$

2.3. Experimental protocol

Animals were randomly divided into six groups: I, II, III, IV, V and VI. Group VI consisted of 10 animals. Each of other groups consisted of 20 animals. Rats in group I were fed basic laboratory chow diet plus 5% micrometer pearl powder for 4 weeks; rats in group II were fed basic laboratory chow diet plus 10% micrometer pearl powder for 4 weeks; rats in group III were fed basic laboratory chow diet plus 5% nanometer pearl powder for 4 weeks; rats in group IV were fed basic laboratory chow diet plus 10% nanometer pearl powder for 4 weeks. Rats in group V were fed basic laboratory chow diet with low content of calcium for 4 weeks; 10 rats in group VI as experimental control were killed before experiment for analysis of femurs weight and length, calcium and phosphorus contents in femurs and serum.

Body weight of rats in groups I–V was monitored periodically (once per week). Three days before concluding the 50-day experiment, calcium content in feces and urine were measured. At the 28th day, rats were sacrificed by cervical dislocation and blood was collected, centrifuged at 1000*g*, 4 °C for 10 min immediately, and then used to assay serum calcium, and phosphorus contents. At the same time, the rats' femur was dissected and cleaned of soft tissue for later analysis of the bone calcium and phosphorus contents of the complete femurs.

2.4. Analysis

2.4.1. Calcium content

Calcium levels in femurs, feces, urine and feedstuff were measured by atomic absorption spectrophotometry (WYX400, ShengYang Analytical instrument Co., Ltd. (Shengyan, China) according to the methodology described by Stewart, Thompson, Furness, and Harrison (1994). Calcium concentrations were presented as $\mu g\ g^{-1}$ (ppm) of tissue on a dry weight basis. Analytical limits of detection were determined as 0.01 $\mu g\ g^{-1}$ dry weight.

Retention rate of calcium and total calcium intake can be calculated according to the method described by Alam, Kabir, Amin, and McNeill (2005). Absorption rate of calcium can be calculated according to the method described by Cui, Yong, Sun, Cao, and Tang (2005).

2.4.2. Femurs weight and length

Femoral length were measured with a caliper made in ShangHai Measuring & Cutting Tool Works (Shanghai, China) and bones were weighed on a precision balance (WP120-1) made in Shanghai Balance Instruments Works (Shanghai, China).

2.4.3. Serum calcium and phosphorus content

Serum calcium level was measured by ethylenediaminetetraacetic acid (EDTA) complex titration (Cem Sayin, Serper, Cehreli, & Kalayci, 2007). Serum phosphorus level was spectrophotometrically determined by molybdenum blue method at 880 nm (Korenaga & Sun, 1996).

2.5. Statistical analysis

Experimental values are presented as the mean ± SD of the number of experiments indicated in the legends. Significance was assessed by using Student's *t*-test ($P < 0.05$ as significant).

3. Results

3.1. Body weight

As for the four weeks feeding study in rats, no abnormal symptoms or deaths had been found in four groups of rats (I–IV) during the experiment. In group V of rats, animals became inactive, and the hair lacked luster and became coarse. We found significant effect of nanometer and micrometer pearl powder on the body weight during the experiment. Body weight at the end of the experiment was significantly higher in the group treated with pearl powder (groups I–IV) ($P < 0.05$, $P < 0.01$) than in group fed low calcium diet (group V) (Table 1). At the end of the experiment the rate of body weight increase of rats in group IV was 230.48%, compared to 171.32%, 197.12% and 218.42% in other three groups treated with pearl powders. In comparison with groups treated with micrometer pearl powder, administration of nanometer pearl powder diet significantly increased the rate of weight increase (%) in groups III and IV in an dose-dependent pattern ($P < 0.05$, $P < 0.01$).

3.2. The effect of nanometer pearl powder on retention and absorption rates of calcium of rats

Retention and absorption rates of calcium of rats were markedly affected by pearl powder feeding (Table 2). Administration of pearl powders significantly increased retention and absorption rates of calcium of rats in groups I–IV in comparison with the experimental control ($P < 0.05$, $P < 0.01$) (group I). Retention and absorption rates of calcium of rats were significantly higher in groups III and IV ($P < 0.05$) compared to those in groups I and II. However, there was significant difference between groups III and IV ($P > 0.05$).

Table 1
The effect of nanometer pearl powder on body weight of rats

Group	*n*	Body weight (g)		Increase rate of body weight (%)
		Before experiment	After experiment	
I	20	77.70 ± 7.27	210.82 ± 30.11[a]	171.32[a]
II	20	76.45 ± 6.19	227.15 ± 34.95[a]	197.12[a]
III	20	74.65 ± 6.04	237.70 ± 39.83[b]	218.42
IV	20	76.60 ± 5.31	253.15 ± 49.24[bc]	230.48[bc]
V	20	79.90 ± 6.39	165.20 ± 24.10	106.70

[a] $P < 0.05$, [b] $P < 0.01$ vs. group V; [c] $P < 0.05$ vs. the two dosages of micrometer pearl powder (groups I and II).

Table 2
The effect of nanometer pearl powder on calcium retention and absorption of rats

Group	*n*	Total calcium absorption (mg/d)	Calcium content in feces (mg/d)	Calcium content in urine (mg/d)	Absorption rate of calcium (%)	Retention rate of calcium (%)
I	20	51.6 ± 9.1	26.5 ± 4.7	6.3 ± 0.9	48.6 ± 9.4[a]	74.9 ± 6.1[a]
II	20	70.4 ± 12.9	30.4 ± 10.3	8.5 ± 1.1	56.8 ± 6.8[a]	78.8 ± 10.2[a]
III	20	53.7 ± 8.3	11.2 ± 2.4	2.1 ± 0.8	79.1 ± 8.3[bc]	95.1 ± 12.3[bc]
IV	20	72.3 ± 13.4	14.1 ± 5.2	3.2 ± 0.4	80.5 ± 8.2[bc]	94.5 ± 9.7[bc]
V	20	10.9 ± 1.6	8.40 ± 2.01	1.9 ± 0.04	22.9 ± 1.6	24.0 ± 2.9

[a] $P < 0.05$, [b] $P < 0.01$ vs. group V; [c] $P < 0.05$ vs. the two dosages of micrometer pearl powder (group I and II).

3.3. The effect of nanometer pearl powder on femurs weight and length of rats

Effects of nanometer pearl powder on femurs weight and length of rats are presented in Table 3. There was a statistically significant effect of pearl powder diet on these bone parameters. The administration of pearl powder diet significantly increased femurs weight and length of rats (groups I–IV), in comparison with the same parameters of rats fed basic laboratory chow diet with low content of calcium (group V) ($P < 0.05$, $P < 0.01$). However, femurs weight and length were heavier and longer ($P < 0.05$, $P < 0.01$) in rats fed nanometer pearl powder diet (groups III and IV), respectively, compared with the same parameters in the groups fed micrometer pearl powder diet (groups I and II). Moreover, the effect of nanometer pearl powder on femurs weight and length of rats exhibited a dose-dependent pattern.

3.4. The effect of nanometer pearl powder on contents of calcium and phosphorus in femurs of rats

Compared with rats fed basic laboratory chow diet with low content of calcium (group V), the pearl powder diets (nanometer and micrometer) significantly dose-dependently increased the contents of calcium and phosphorus, retention rate of calcium, ratio of calcium to phosphorus in femur of rats in groups I–IV ($P < 0.05$, $P < 0.01$) (Table 3). Moreover, these indices in femur of rats fed the nanometer pearl powder diets (groups III and IV) were significantly higher than those in rats fed micrometer pearl powder diet (groups I and II) ($P < 0.05$, $P < 0.01$).

3.5. The effect of nanometer pearl powder on contents of calcium and phosphorus in serum of rats

The contents of calcium and phosphorus in serum of rats fed pearl powder diets are shown in Table 4. The rats fed pearl powder diets (groups I–IV) demonstrated higher

Table 3
The effect of nanometer pearl powder on femurs weight and length of rats and contents of calcium and phosphorus in femurs

Group	n	Femurs length (cm)	Femurs weight (g)	Total calcium absorption (g)	Calcium in femurs (mg)		Retention rate of calcium (%)	Phosphorus in femurs (mg)	Calcium to phosphorus ratio
					Before experiment	After experiment			
I	20	2.58 ± 0.11^{a}	0.75 ± 0.09^{a}	2.24 ± 0.34	53.36 ± 5.34	83.32 ± 11.34^{a}	1.34^{a}	45.05 ± 3.45	1.85^{a}
II	20	2.67 ± 0.09^{a}	0.81 ± 0.07^{a}	2.69 ± 0.47	53.36 ± 5.34	94.36 ± 13.45^{b}	1.52^{b}	50.29 ± 3.54	1.88^{a}
III	20	2.81 ± 0.12^{bc}	0.95 ± 0.14^{bc}	2.21 ± 0.53	53.36 ± 5.34	120.41 ± 12.14^{bc}	3.03^{bc}	61.31 ± 8.51	1.96^{bc}
IV	20	2.97 ± 0.18^{bd}	1.03 ± 0.11^{bd}	2.73 ± 0.31	53.36 ± 5.34	147.49 ± 17.62^{bc}	3.45^{bd}	70.39 ± 8.99	2.09^{bc}
V	20	2.41 ± 0.04	0.57 ± 0.06	0.74 ± 0.01	53.36 ± 5.34	47.86 ± 8.98	0.74	30.73 ± 7.58	1.33

[a] $P < 0.05$, [b] $P < 0.01$ vs. group V; [c] $P < 0.05$, [d] $P < 0.01$ vs. the two dosages of micrometer pearl powder (groups I and II).

Table 4
The effect of nanometer pearl powder on contents of calcium and phosphorus in serum of rats

Group	n	The content of calcium in serum (mmol/L)	The content of phosphorus in serum (mmol/L)	The calcium to phosphorus ratio
I	20	1.97 ± 0.23^{a}	2.05 ± 0.11	0.96
II	20	2.17 ± 0.28^{a}	2.06 ± 0.33	1.05
III	20	2.36 ± 0.21^{bc}	2.31 ± 0.15^{b}	1.02
IV	20	2.59 ± 0.40^{bc}	2.45 ± 0.37^{b}	1.06
V	20	1.82 ± 0.19	1.91 ± 0.21	0.95
VI	10	2.08 ± 0.27	1.99 ± 0.34	1.05

[a] $P < 0.05$, [b] $P < 0.01$ vs. group V; [c] $P < 0.05$, [d] $P < 0.01$ vs. the two dosages of micrometer pearl powder (groups I and II).

contents of serum calcium and phosphorus and were significantly different ($P < 0.05$, $P < 0.01$) from those fed basic laboratory chow diet with low content of calcium (group V). Moreover, higher contents of calcium and phosphorus ($P < 0.05$, $P < 0.01$) were observed in serum of rats fed nanometer pearl powder diet (groups III and IV) in comparison with rats fed micrometer pearl powder diet (groups I and II). Furthermore, the contents of calcium and phosphorus in rats fed 10% nanometer pearl powder diet was greater than those fed 5% nanometer pearl powder diet.

Table 5
Calcium increase in test samples and their increase percentage

Group	n	Calcium[a] increase in feces (mg/d)	Increase[b] percentage (%) in feces	Calcium[a] increase in urine (mg/d)	Increase[b] percentage (%) in urine	Calcium[a] increase in femurs (mg)	Increase[b] percentage (%) in femurs	Calcium[a] increase in serum (mmol/L)	Increase[b] percentage (%) in serum
I	20	18.1	215	4.4	232	35.46	74	0.15	8
II	20	22	262	6.6	347	46.5	97	0.35	19
III	20	2.8	33	0.2	11	72.55	152	0.54	30
IV	20	5.7	68	1.3	68	99.36	208	0.77	42
V	20	(8.40)		(1.9)	232	(47.86)	74	(1.82)	8

[a] Calcium increase in faces = calcium content in treatment groups (I–IV) − calcium content in control group (V).
[b] Calcium increase in faces = (calcium content in treatment groups (I–IV) − calcium content in control group (V))/calcium content in control group.

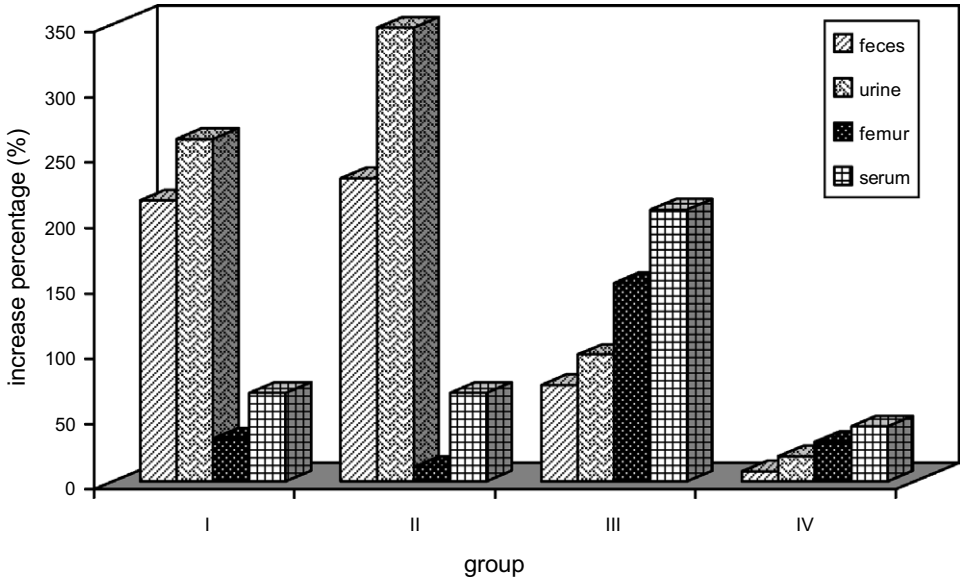

Fig. 1. Increase percentage of calcium in test samples.

3.6. The effect of nanometer pearl powder on increase percentage of calcium in test samples of rats fed pearl powder diets

The calcium increase and its increase percentage in test samples (feces, urine, femur and serum) of rats fed pearl powder diets (I–IV) are shown in Table 5 and Fig. 1. Rats fed micrometer pearl powder diets (groups I and II) demonstrated a 215% and 262% increase percentage in feces, respectively, a 232% and 347% increase percentage in urine, respectively, while rats fed nanometer pearl powder diets (groups III and IV) demonstrated a 33% and 68% increase percentage in feces, respectively, a 11% and 68% increase percentage in urine, respectively, in comparison with those fed basic laboratory chow diet with low content of calcium (group V). In addition, the rats fed micrometer pearl powder diets (groups I and II) demonstrated a 74% and 97% increase percentage in femur, respectively, a 8% and 19% increase percentage in serum, respectively, while the rats fed nanometer pearl powder diets (groups III and IV) demonstrated a 152% and 208% increase percentage in femur, respectively, a 30% and 42% increase percentage in serum, respectively, in comparison with those fed basic laboratory chow diet with low content of calcium (group V). These results indicated that calcium absorption rate increase with decreased particle size of calcium.

4. Discussion

Calcium is an essential mineral found in great abundance in the body. It is necessary for allowing a bone mineral accretion adequate for skeletal growth and maturation (Heaney, Recker, & Savillep, 1977). Ninety-nine percent of all the calcium in the body is found in the bones and teeth. The remaining 1% is in the blood. Calcium also plays important roles in nerve conduction, muscle contraction, and blood clotting (Kim et al., 2005; Recker & Heaney, 1985). If calcium levels in the blood drop below normal, calcium will be taken from bone and put into the blood in order to maintain blood calcium levels. Therefore, it is important to consume enough calcium to maintain adequate blood and bone calcium levels. Dolomite, pearl, and bone meal are naturally occurring calcium carbonate sources. Different sizes of pearl powders have been developed for calcium supplement.

The results in this paper demonstrate differences in the properties of calcium release from nanometer pearl powder and micrometer pearl powder. The effect of absorption and utilization of calcium released from the two sizes of pearl powders in rats are summarised in Tables 1–5. It is interesting that absorption and utilization of calcium in rats differ with difference of sizes of pearl powders. The results of the work strongly support the favorable effect of the two sizes of pearl powders on absorption and utilization of calcium and bone formation in rats. However, it is noticeable that the effect of nanometer pearl powder on absorption and utilization of calcium and bone formation in rats was markedly better than micrometer pearl powder. Therefore, this study strongly supports that the smaller size of pearl powders could effectively reduce difficulty of calcium release, increase absorption of calcium and maintain bone normal function in rats. A possible explanation is that effect of particle sizes of mineral materials on digestibility and solubility is an important factor to affect their bioavailability. If mineral materials in alimentary tract are insoluble, they can not be absorbed. Therefore, when mineral materials were utilized to supply microelement, particle sizes were very important. When pearl powders were reduced to at least micrometer sizes, its bioavailability was greatly increased.

In addition, we have found that rats fed pearl powders diet had increased phosphorus content in femurs except rats fed 10% nanometer pearl powders diet. Although feces and urinary calcium excretion may be enhanced by increasing calcium intake, calcium content in urine and feces of rats in our study did not significantly increase with the increase of calcium intake and bioavailability. These results disagree with the observations of others (Kansal & Chaudhary, 1982; Ranhotra, Lee, & Gelroth, 1980). It is well documented that high intakes of calcium can depress absorption of magnesium, phosphorus and zinc in some situations (Greger, 1982; Greger, Smith, & Snedekers, 1981; Monsen & Cook, 1976). This is also supported by our results. In the present experiment, phosphorus content in femurs and serum of rats fed 10% nanometer pearl powders diet is the lowest. Hence fortification of calcium supplements with magnesium, phosphorus etc is reasonable. Moreover, calcium, as judged by retention rate of calcium, appeared to be affected by the bioavailability of calcium fed to rats in the study. A basic premise in balance studies is that increased apparent absorption of calcium and/or decreased urinary losses of calcium lead to a favorable outcome-increased calcium retention.

In general, our data suggest that nanometer pearl powders possess higher bioavailability and security, and may be used as calcium supplements in food and medicine.

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