

Letter to the Editor

Effect of Different Calcium Supplements on Bone Metabolism in Rats

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Osteoporosis, characterized by loss of bone mass and microarchitectural deterioration of bone tissue, results in enhanced bone fragility and increases risk of fracture<sup>[1]</sup>. In China, the incidence of primary osteoporosis is as high as 50%-70% in 60-69 years old females and approximately 30% in 60-69 years old males<sup>[2]</sup>, which is closely related with the low intake of calcium. According to the nationwide nutrition and health survey in 2002 in China, the average daily calcium intake of Chinese residents is 391 mg, accounting for 41% of the recommended calcium intake<sup>[3]</sup>. Therefore, urgent measures should be taken to improve the insufficient calcium intake in this population. Considering the low absorption rate of calcium in natural Chinese foods, calcium supplement is an optional method. At present, different kinds of calcium supplements are available in pharmacy sales and people often do not know how to choose them. Six calcium supplements, including nanometer calcium carbonate (NCC), microcrystal calcium hydroxyapatite (MCH), whey calcium (WC), enzymatic cattle bone powder (ECBP), ultramicro enzymatic cattle bone powder (UECBP), and enzymatic fishbone powder (EFBP), were therefore selected in this study and their effects on bone metabolism in rats were compared in order to provide scientific evidence for proper selection of calcium supplements in general public.

NCC is an ultrafine calcium carbonate powder with a particle size of 1-100 nm, which can thus help to enlarge its contact area with digestive juice<sup>[4]</sup>. Calcium from animal sources mainly exists in the form of crystal hydroxyapatite and is hard to be absorbed by humans due to its low solubility. MCH is an ultrafine crystal hydroxyapatite calcium. Protease can dissolve the remaining protein in bone powder to help release of calcium. Released calcium further interacts with amino acids to form soluble amino acid-chelated calcium, which can be easily

absorbed<sup>[5-6]</sup>. The sources of enzymatic bone powder used in this study were yellow cattle and sea-fish. WC is a concentrate of whey mineral salt extracted directly from milk. Its proper calcium/phosphorus ratio (2:1) and richness in lactose and protein can significantly increase calcium absorption.

One hundred and fifty female SD rats weighing 60-75 g were purchased from Department of Laboratory Animal Science, Peking University. The study was approved by the Animal Care and Use Committee of Peking University. The rats were randomly divided into 12 calcium supplement treatment groups, 2 relative calcium carbonate (CC) control groups and 1 low calcium (LC) control group (10 in each group) and fed with different animal foods produced by Institute of Laboratory Animals, Chinese Academy of Medical Sciences. Rats in LC control group were fed with basic rat foods containing 150 mg/100 g calcium. Those in calcium supplement and CC groups were fed with rat foods containing 500 mg/100 g and 1000 mg/100 g calcium. The rats were housed in plastic cages (1 per cage) at 23-24 °C with a relative humidity of 54%-58%. All rats had free access to foods but no to deionized water.

Body weight and body length were measured and food intake was determined once a week. Food utilization was calculated according to the following equation (1).

$$\text{Food utilization (\%)} = \frac{\text{animal weight gain (g)}}{\text{food intake (g)}} \times 100\% \tag{1}$$

Bone metabolism was tested for 3 days at the end of week 3. Calcium in feces and food samples was collected and analyzed by ICP-OES (icap-6000, Thermo Fisher Scientific, USA). Calcium apparent absorption rate was computed according to the following equations 2-4.

$$\text{Calcium intake (mg/3d)} = \text{feed calcium content (mg/g)} \times \text{feed intake (g/3d)} \tag{2}$$

doi: 10.3967/0895-3988.2013.08.007  
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Feces calcium (mg/3d) = feces calcium content (mg/g) × feces dry weight (g/3d) (3)

Calcium apparent absorption rate (%) = 
$$\frac{\text{calcium intake} - \text{feces calcium}}{\text{calcium intake}} \times 100\%$$
 (4)

The experiment was terminated after 13 weeks of feeding. Blood and femur samples were collected and calcium in the right femur bone was assayed by ICP-OES. Bone mineral density (BMD) was measured by DEXA (NORLAND XR-36, Lunar Corp., USA) at the midpoint, the proximal and distal end of left femur bone. Serum ALP level was measured using P-nitrophenol phosphate method, and blood calcium level was measured by AAS. Twelve-hour urine samples were collected after 12 weeks of feeding. Hydroxyproline and creatinine levels were then measured by ultraviolet-visible spectroscopy (FR-200, Xiari Science Corp., Shanghai, China). All data were analyzed by ANOVA using the SPSS software version 13.0.

The body weight was significantly higher in low UECBP group than in CC group ( $P<0.05$ ) whereas the body length was significantly lower in low NCC group than in CC group after 13 weeks of feeding with no significant difference observed in the total food utilization during the 13 week feeding period ( $P>0.05$ , Table 1).

The calcium apparent absorption rate (CAAR) was significantly lower in 12 treatment groups than in LC group ( $P<0.05$ ) probably due to the compensatory regulation mechanism in rats with severe calcium deficiency. The CAAR was significantly lower in MCH groups and high WC, ECBP, UECBP, and EFBP groups than in CC group ( $P<0.05$ ) with no significant difference observed in NCC groups ( $P>0.05$ ). The CAAR was higher in NCC groups (68.16% and 61.30%) among 12 treatment groups and significantly higher in high ECBP and UECBP groups than in relative EFBP group, indicating that the calcium from yellow cattle bone can be more easily absorbed than from sea-fish bone (Figure 1).

**Table 1.** Effect of Calcium Supplements on Growth of Rats (Mean±SD)

Group		Feed Calcium Content (mg/100 g)	Number	Body Weight (g)		Body Length (cm)		Food Utilization (%) <sup>*</sup>
				Week 0	Week 13	Week 0	Week 13	
NCC	Low dosage	500	10	66.5±4.6	317.5±53.9	24.4±0.8	39.0±1.8 <sup>£</sup>	18.0±2.1
	High dosage	1000	10	65.9±5.2	307.9±26.9	24.6±1.1	39.5±1.5	17.2±0.9
MCH	Low dosage	500	10	64.9±3.6	328.0±37.4	24.7±1.1	39.7±1.4	17.5±1.2
	High dosage	1000	10	66.0±4.5	320.6±36.4	24.5±1.2	39.8±1.6	17.5±2.3
WC	Low dosage	500	10	65.8±4.7	339.4±34.5 <sup>¶</sup>	24.6±1.4	39.7±1.0	19.0±1.3
	High dosage	1000	10	66.1±3.8	347.3±32.9 <sup>¶</sup>	24.4±0.9	40.5±1.9	19.0±1.1
ECBP	Low dosage	500	10	66.6±3.0	340.9±36.3 <sup>¶</sup>	24.4±0.5	40.6±1.5	18.3±1.2
	High dosage	1000	10	64.6±4.2	329.6±51.7	24.3±1.1	39.6±1.2	17.9±1.7
UECBP	Low dosage	500	10	67.2±4.1	347.7±35.5 <sup>¶£</sup>	24.3±1.0	40.3±1.2	19.0±0.8
	High dosage	1000	10	68.1±5.1	330.0±22.6	24.0±1.4	40.6±1.7	18.0±0.9
EFBP	Low dosage	500	10	67.7±4.2	336.6±29.7	24.0±0.9	40.4±1.2	17.8±1.2
	High dosage	1000	10	67.5±3.2	335.4±42.6	24.1±0.4	40.3±1.6	17.4±1.7
CC	Low dosage	500	10	67.8±4.4	309.5±26.9	24.4±1.2	40.4±0.9	17.2±1.5
	High dosage	1000	10	66.4±3.8	326.0±33.7	24.3±1.2	40.1±1.4	17.2±1.0
LC	control group	150	10	67.8±4.4	306.2±36.6	24.1±1.0	39.7±1.1	17.3±1.6

**Note.** <sup>¶</sup> vs LC group ( $P<0.05$ ), <sup>£</sup> vs relative CC group ( $P<0.05$ ), <sup>\*</sup> total utilization of food in 13 weeks. NCC: nanometer calcium carbonate; MCH: microcrystal calcium hydroxyapatite; WC: whey calcium; ECBP: enzymatic cattle bone powder; UECBP: ultramicro enzymatic cattle bone powder; EFBP: enzymatic fishbone powder; CC: calcium carbonate; LC: low calcium.

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