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[1]. 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[2], 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[3]. 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[4]. 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[5-6]. 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).

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

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.

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

doi: 10.3967/0895-3988.2013.08.007

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$$\text{Feces calcium (mg/3d)} = \text{feces calcium content (mg/g)} \times \text{feces dry weight (g/3d)} \quad (3)$$

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

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 calciumlevel 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 (%)*
				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[£]	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[¶]	24.6±1.4	39.7±1.0	19.0±1.3
	High dosage	1000	10	66.1±3.8	347.3±32.9[¶]	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[¶]	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[¶£]	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. [¶]*vs* LC group (P<0.05), [£]*vs* relative CC group (P<0.05), *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.

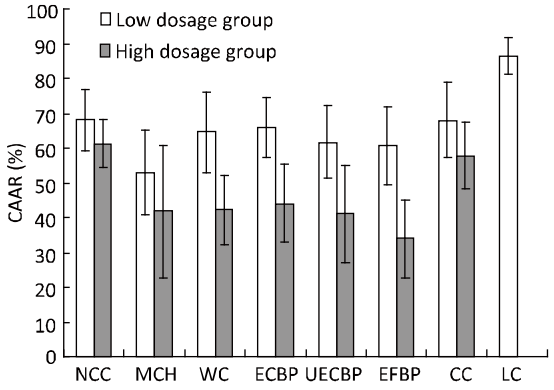

Figure 1. Effect of calcium supplements on CAAR in rats. NCC: nanometer calcium carbonate; MCH: microcrystal calcium hydroxyapatite; WC: whey calcium; ECBP: enzymatic cattle bone powder; UECBP: ultramicro enzymatic cattle bone powder; EFBP: enzymatic fishbone powder; CC: calcium carbonate; LC: low calcium.

The left femur BMD was significantly higher in 12 treatment groups than in LC control group (P<0.05) and was higher in MHC, WC, UECBP, and EFBP groups than in CC group (P<0.05), the BMD was lower in high ECBP group at the femur distal end (0.311 g/cm^2) than in CC group (P<0.05). The bone calcium content (BCC) was significantly higher in ECBP and EFBP groups and high WC group than in LC group (P<0.05). The BCC was significantly lower in NCC, MCH, and UECBP groups and low WC group than in relative CC group (P<0.05, Table 2).

CAAR, BMD, and BCC are usually used in animal experiments to determine whether the tested calcium supplement can improve BMD. However, the CAAR is usually affected by certain factors, such as calcium nutritional status, vitamin D, and sports level[7]. DEXA, as the golden standard for the BMD, originally designed for humans, can result in biggish errors when it is used in animals, exhibiting a poor repeatability if the variance between groups is small.

Table 2. Effect of Calcium Supplements on Femur Bone in Rats (Mean±SD)

Group		Number	BMD at Femur Midpoint (g/cm^2)	BMD at Femur Distal End (g/cm^2)	BMD at Femur Proximal End (g/cm^2)	Femur Bone Length (mm)	Bone Calcium Content (mg/g)	Bone Constant Weight (mg)
NCC	Low dosage	10	0.219±0.010¶	0.312±0.042¶	0.256±0.012¶	34.62±1.18	257.0±17.0$^{£}$	549.0±43.1¶
	High dosage	10	0.217±0.014¶	0.317±0.013¶	0.260±0.009¶	34.54±0.53$^{£}$	256.8±24.7$^{£}$	542.3±39.2$^{¶£}$
MCH	Low dosage	10	0.229±0.006¶	0.315±0.017¶	0.264±0.010$^{¶£}$	34.78±0.67	264.9±7.3	548.2±38.9¶
	High dosage	10	0.232±0.012¶	0.332±0.024¶	0.275±0.012$^{¶£}$	34.86±0.78	253.9±12.2$^{£}$	587.0±55.0¶
WC	Low dosage	10	0.230±0.012¶	0.330±0.020¶	0.267±0.013$^{¶£}$	34.71±0.73	256.8±10.1$^{£}$	571.0±48.9¶
	High dosage	10	0.245±0.010$^{¶£}$	0.318±0.015¶	0.278±0.009$^{¶£}$	35.33±1.19¶	278.9±7.0¶	587.1±47.2¶
ECBP	Low dosage	10	0.227±0.012¶	0.319±0.017¶	0.269±0.012$^{¶£}$	34.69±0.50	277.8±4.3¶	574.7±31.6¶
	High dosage	10	0.234±0.014$^{¶£}$	0.311±0.018$^{¶£}$	0.271±0.020¶	34.71±0.90	277.6±10.5¶	555.1±39.6¶
UECBP	Low dosage	10	0.233±0.010¶	0.325±0.020¶	0.276±0.010$^{¶£}$	35.13±0.42	264.4±8.6$^{£}$	592.1±28.7¶
	High dosage	10	0.231±0.013¶	0.332±0.019¶	0.272±0.011¶	34.88±0.80	261.2±11.9$^{£}$	591.4±52.6¶
EFBP	Low dosage	10	0.231±0.006¶	0.320±0.016¶	0.265±0.012$^{¶£}$	35.14±0.95	279.7±8.0¶	593.7±41.5¶
	High dosage	10	0.233±0.011¶	0.322±0.020¶	0.264±0.015¶	35.59±0.66¶	287.2±13.0¶	594.3±57.9¶
CC	Low dosage	10	0.222±0.011¶	0.319±0.023¶	0.253±0.014¶	35.24±1.01	286.1±7.8¶	572.5±49.9¶
	High dosage	9	0.225±0.009¶	0.332±0.018¶	0.264±0.007¶	35.53±1.01¶	287.0±9.0¶	589.5±44.6¶
LC	control group	10	0.181±0.009	0.251±0.015	0.212±0.011	34.45±1.83	254.2±15.5	442.9±42.9

Note. ¶ *vs* LC group(P<0.05); $^{£}$ *vs* relative CC group (P<0.05). 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.

BCC, as another evaluation index, is not used as often as BMD. In this study, the conclusion drawn according to BCC is inconsistent with that drawn according to BMD. For example, the femur BCC was lower while the femur BMD was higher in low MCH and WC groups than in CC group probably due to the disadvantages of DEXA in measuring the BMD of animals. It is, therefore, recommended that BCC should be regarded as the major indicator while BMD as the minor indicator in evaluating the effect of calcium supplements on bone metabolism[8], which has not been universally accepted and needs further confirmation.

The blood calcium level was significantly lower in high UECBP group and EFBP groups than in relative CC group (P<0.05) whereas the serum ALP level was significantly higher in high NCC and MCH groups than in CC group at week13 (P<0.05, Table 3).

In conclusion, the BCC and BMD are significantly higher in NCC groups than in LC control group with no significant difference observed in BCC, BMD, and CAAR between NCC groups and relative CC group. However, the CAAR for the remaining 5 calcium supplements is significantly lower than relative CC group, indicating that NCC is a better choice for calcium supplement.

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Received: September 20, 2012;

Accepted: April 25, 2013

Table 3. Effect of Calcium Supplements on Bone Metabolism in Rats (Mean±SD)

Group		Serum ALP Level (U/L)		Blood Calcium Concentration (mmol/L)		Urine Hydroxyproline and Creatinine Ratio (×10^{-3})
		4th week	13th week	4th week	13th week	13th week
NCC	Low dosage	288.1±64.6[£]	141.7±35.2	2.43±0.14	2.47±0.13[¶]	14.0±8.9
	High dosage	303.2±85.7[£]	154.0±62.6[¶£]	2.63±0.12[¶]	2.55±0.08[¶]	11.5±7.1
MCH	Low dosage	276.3±31.2[£]	137.1±40.9	2.47±0.08[¶]	2.42±0.09	9.4±3.7
	High dosage	244.0±75.8	164.8±81.5[¶£]	2.45±0.07[¶£]	2.53±0.12[¶]	9.3±4.8
WC	Low dosage	262.4±64.2	140.3±46.8	2.42±0.10	2.45±0.12	11.8±6.7
	High dosage	219.6±39.2	115.0±19.7	2.37±0.09[£]	2.44±0.09	8.0±5.5
ECBP	Low dosage	241.5±84.2	143.2±41.6	2.35±0.10	2.47±0.12[¶]	12.7±4.9
	High dosage	236.7±54.8	129.2±26.0	2.36±0.10[£]	2.37±0.12	13.8±11.7
UECBP	Low dosage	241.4±59.9	108.0±36.7	2.35±0.13	2.40±0.11	11.2±5.6
	High dosage	270.8±97.2	103.8±34.8	2.31±0.09[£]	2.35±0.11[£]	7.6±6.0
EFBP	Low dosage	215.3±57.1	89.0±38.7	2.38±0.11	2.29±0.17[£]	9.8±6.5
	High dosage	213.7±54.7	92.0±25.9	2.37±0.07[£]	2.26±0.17[£]	5.9±2.5[¶]
CC	Low dosage	217.3±53.4	109.2±31.2	2.39±0.08	2.41±0.12	10.4±6.6
	High dosage	242.6±54.3	103.4±29.9	2.55±0.11[¶]	2.46±0.12	10.7±2.4
LC	control group	265.7±34.9	113.4±25.7	2.36±0.09	2.35±0.13	14.4±10.8

Note. [¶] *vs* LC group (P<0.05); [£] *vs* relative CC group (P<0.05). 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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