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Comparison of Limestone and Ground Fish for Treatment of Nutritional Rickets in Children in Nigeria

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Objective To determine whether children with calcium-deficiency rickets respond better to treatment with calcium as limestone or as ground fish.

Study design Nigerian children with active rickets (n = 96) were randomized to receive calcium as powdered limestone (920 mg of elemental calcium) or ground fish (952 mg of elemental calcium) daily for 24 weeks. Radiographic healing was defined as achieving a score of 1.5 or less on a 10-point scale.

Results The median (range) age of enrolled children was 35 (6-151) months. Of the 88 children who completed the study, 29 (66%) in the ground fish group and 24 (55%) in the limestone group achieved the primary outcome of a radiographic score of 1.5 or less within 6 months ($P = .39$). The mean radiographic score improved from 6.2 ± 2.4 to 1.8 ± 2.2 in the ground fish group and from 6.3 ± 2.2 to 2.1 ± 2.4 in the limestone group ($P = .68$ for group comparison). In an intention to treat analysis adjusted for baseline radiographic score, age, milk calcium intake, and serum 25-hydroxyvitamin D concentration, the response to treatment did not differ between the 2 groups ($P = .39$). Younger age was associated with more complete radiographic healing in the adjusted model (aOR 0.74 [95% CI 0.57-0.92]). After 24 weeks of treatment, serum alkaline phosphatase had decreased, calcium and 25-hydroxyvitamin D increased, and bone mineral density increased in both groups, without significant differences between treatment groups.

Conclusion In children with calcium-deficiency rickets, treatment with calcium as either ground fish or limestone for 6 months healed rickets in the majority of children. *(J Pediatr 2015;167:148-54)*.

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Although nutritional rickets has been described classically as a disease caused by vitamin D deficiency, inadequate calcium intake may be a more important cause in tropical countries, including Nigeria. Several studies have shown that calcium supplementation for 6 months effectively heals rickets in the majority of Nigerian children.[1-4] The cost of calcium tablets (approximately US $12 per month), however, is beyond the reach of many families in low-income countries. The effectiveness of less costly, indigenous forms of calcium supplementation in treating rickets has not been explored.

Dairy products provide most of the dietary calcium of children in high-income countries but often are unaffordable and unavailable in developing countries. The average intake of calcium in the diet of Nigerian children is 200 mg,[5] far less than the estimated average requirement recommended for North American children of 500 mg in children ages 1-3 years and 800 mg in children ages 4-8 years.[6]

Dried fish is a component of the diet in many low-income countries, including Nigeria, and the bones of fish are an excellent source of calcium, which in studies in animals has been shown to be retained as effectively as milk calcium.[7] After dried fish are ground, the bones may be consumed without the risk of lodging in the throat or esophagus. Because it is already a component of the diet, dried fish may be a suitable source of calcium supplementation in low-income countries.

Alternatively, limestone is an abundant form of calcium carbonate that contains approximately 30% elemental calcium. The use of limestone in the diet is not new; limestone is used in Mexico, where rickets is rare, in the preparation of corn tortillas to facilitate removal of the outer coating of the corn.[8] Because of its abundance and minimal cost, limestone may be an appropriate source of calcium supplementation in low-income countries. Our objective was to test whether ground fish and limestone would be equally effective for treatment of rickets caused by dietary calcium deficiency in Nigerian children.

25(OH)D	Serum 25-hydroxyvitamin D
aBMD	Areal bone mineral density

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Methods

Children with deformities characteristic of rickets (genu varum, genu valgum, widened wrists) were recruited as they presented to the outpatient department of the Jos University Teaching Hospital in Jos, Nigeria. Each child was examined, and a parent was interviewed. Children with clinical evidence of rickets underwent radiography of the wrists and knees. We used a 10-point radiographic scale to measure the severity of radiographic changes in the wrists and knees[9]; a score of 10 indicates the most severe radiographic features of rickets. Rickets was considered active, and children were eligible for enrollment if they had a radiographic score of 2 or greater. Children were excluded if they had taken vitamin D or calcium supplements in the preceding 4 weeks. Only children who were at least 6 months of age were enrolled in the study.

Participation was voluntary, and written informed consent was obtained from the parent or guardian of each child. The study was approved by the Ethical Committee of the Jos University Teaching Hospital and the Institutional Review Board of the Mayo Clinic.

We collected information about family size, birth order, breast-feeding history, usual dairy product intake, and religion, education, and occupation of the parents. Parents were not instructed to modify their child's diet during the study but were encouraged to provide the child with regular exposure to sunlight for vitamin D. Standing height and weight were measured at baseline, 12, and 24 weeks. Z-scores of weight for height, weight for age, and height for age, which permit comparison of nutritional status across age and sex groups, were calculated with the nutritional anthropometric program of Epi Info version 6.04c (Centers for Disease Control and Prevention, Atlanta, Georgia).

Radiographs of wrists and knees were repeated at 12 and 24 weeks after the children started treatment. Three physicians who were unaware of treatment group assignment and the temporal sequence of radiographs independently scored each radiograph. The mean value of the 3 scores was used for the analysis.

Eligible children were assigned randomly in blocks of 10 to receive fish meal or limestone for a period of 24 weeks. We used sealed envelopes for allocation concealment. Children assigned to the ground fish group received a 4-weekly supply (10 g twice daily) of ground fish, ground and prepared by the Jos University Teaching Hospital Dietetics Department from baked local species of dried catfish (*Clarias gariepinus* or *Heterobranchus longifilis*). The cost of ground fish varied between US $1 and $2 for a 1-month supply. Ground fish commonly is prepared with a food grinder used in local marketplaces.

Children assigned to the limestone group were given a 4-weekly supply (1.75 g twice daily) of powdered limestone (5-μm diameter particle size). Powdered limestone was available commercially in 50-kg bags used in the production of toothpaste and cosmetics, and the cost of 6 months of treatment was approximate US $0.08. In comparison, calcium supplements available in Nigerian pharmacies cost about US $190 for 6 months of treatment. On the basis of analysis of 18 monthly samples, the elemental calcium content of ground fish was 4.6 g per 100 g, and that of limestone 27.2 g per 100 g. Thus, the assigned quantities of ground fish and limestone provided 920 and 952 mg of elemental calcium daily, respectively.

Parents were instructed to mix the limestone or ground fish with the child's food. The ground fish and limestone had no toxic concentrations of heavy metals (US Department of Agriculture. Agricultural Research Service US Plant, Soil & Nutrition Laboratory, Cornell University, Ithaca, New York). Children returned every 4 weeks to collect a new supplement and to be monitored for adverse effects. To assess the child's compliance with the study, parents were instructed to bring the remaining supplement to be weighed with an analytical triple-beam pan weighing scale.

Biochemical Measurements

Serum samples were obtained from the children at baseline and at 12 and 24 weeks. All samples were stored at −20°C until transported on dry ice to the Mayo Clinic for biochemical testing. Serum calcium, phosphorus, and albumin were measured using standard methods. The method of analysis of alkaline phosphatase changed during the study. Of the 213 alkaline phosphatase samples, 108 were analyzed before June 16, 2003 with a diethanolamine buffer, and 105 later samples were analyzed with a 2-amino-2-methyl-1-propanol buffer. On the basis of laboratory validation data, values using the older method were multiplied by 0.46 to correspond to values comparable with newer method. All time points for a single subject were analyzed together with the same method, so that the change in alkaline phosphatase with treatment was independent of the method used. Serum 25-hydroxyvitamin D [25(OH)D] was measured by isotope-dilution liquid chromatography tandem mass spectrometry.[10]

Bone Densitometry

Dual-energy X-ray absorptiometry of the left radius and ulna was performed by a single investigator (T.T.) with a pDEXA portable bone densitometer (Model 476A110; Norland Products Inc, Cranbury, New Jersey). Measurement sites included the area of minimal areal bone mineral density (aBMD) of the distal radius and ulna (primarily metaphyseal bone) and the proximal one-third of the radius and ulna (primarily diaphyseal bone). The instrument was set at standard precision and calibrated daily. To minimize movement artifact, the child was laid supine on a custom-made platform with his or her arm secured by Velcro straps, and the hand and elbow were held by an assistant to minimize movement. The parent was present to reassure the child. Most study subjects were cooperative and relatively motionless. If the investigator judged that excessive motion artifact was present, the scan was repeated. If the second attempt was unsuccessful, the bone density data were excluded for that visit.