KASHIN-BECK DISEASE: EVALUATION OF MINERAL INTAKE IN YOUNG TIBETAN CHILDREN FROM ENDEMIC AREAS

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Objectives

The main objective of this work was to measure the mineral content of most consumed Tibetan foods and to compare it to international reference tables. Highlight excess or deficiency among young Tibetan children from endemic areas was a secondary objective. 24-hour food recalls were collected by the mean of prospective nutritional questionnaire. Mineral daily intakes were evaluated combining foods mineral contents and 24-hour food recalls .

Introduction

Kashin-Beck Disease

Kashin-Beck disease (KBD) is an endemic and chronic osteochondropathy characterized by short stature and skeletal deformities especially in long bones and joints. Joints become enlarged, stiff and painful. Mobility of limbs become limited and muscles can be atrophied. Peoples suffering from KBD get tired quickly and are weaker. Symptoms appear during childhood (3-5 years) and get progressively worse.

This disease principally occurs in the Tibet Autonomous Region and in several provinces of the People's Republic of China.

Although many studies have already been conducted and many others are still underway, its ethiology remains unknown. A multifactorial hypothesis has been proposed:

- selenium deficiency;
- high concentration of organic matters in drinking water (fulvic acids);
- mycotoxin poisoning by fungi infecting cereals.

Minerals and bone metabolism

Dozen of mineral elements directly or indirectly step in cartilage and bone metabolisms (chondrogenesis, bone osteogenesis, bone growth and bone homeostasis).

Recommended dietary intakes depend on several factors such as metabolic needs, life stage group and bioavailability.

Table 1 Recommended Dietary Allowances (RDAs) and Adequate Intakes (AIs) for the concerned life stage groups investigated in the present study. Issued from the *Institute of Medicine of the National Academies.*

Life Stage Group	**P** (mg/d)		**Ca** (mg/d)		**Mg** (mg/d)		**Fe** (mg/d)	
	RDA/AI*	UL	RDA/AI*	UL	RDA/AI*	UL	RDA/AI*	UL
1-3 years	460	3000	500*	2 500	80	65	7	40
4-8 years	500	3000	800*	2 500	130	110	10	40
	Zn (mg/d)		**Cu** (µg/d)		**Mn** (mg/d)		**Se** (µg/d)	
	RDA/AI*	UL	RDA/AI*	UL	RDA/AI*	UL	RDA/AI*	UL
1-3 years	3	7	340	1 000	1.2*	2	20	90
4-8 years	5	12	440	3 000	1.5*	3	30	150

RDAs are set to meet the needs of almost all individuals in a group.
AIs are believed to cover needs of all individuals in the group, but lack of data or uncertainty in the data prevent being able to specify with confidence the percentage of individuals covered by this intake. Number followed by * refer to AIs.
UL (upper limit) is the maximum level of daily nutrient intake that is likely to pose no risk of adverse effects. ULs for magnesium represent intake from a pharmacological agent only and do not include intake from food and water.
ND = not determinable.

Table 2 Most important dietary minerals involved in bone metabolism, their main functions and adverse effects of deficiency or excessive consumption.

Chemical element	Main functions	Adverse effects of deficiency or excessive consumption
Phosphorus (P)	Constituent of bone inorganic matrix (hydroxylapatite). Essential for enzymatic reactions involved in bone mineralization.	Long-term P excess combined with Ca deficiency could have negative impact on bone mineralization and remodelling .
Calcium (Ca)	Constituent of bone inorganic matrix (hydroxylapatite).	Long term calcium deficiency (or vitamin D) leads to insufficient mineralisation of ossein in children (rickets).
Magnesium (Mg)	Constituent of bone inorganic matrix (hydroxylapatite). Cofactor of many enzymes involved in bone metabolism.	Persisting hypomagnesaemia could induce hypoparathyroidism and disturbance of the vitamin D metabolism.
Iron (Fe)	Cofactor of two important enzymes involved in bone formation (*procollagen-proline hydroxylase* and *procollagen-lysine hydroxylase).*	Rats fed with Fe deficient diet present low bone mineral density and low bone mineral content. Iron excess inhibits osteoblast activity (decrease of mineralization). Iron excess can increase oxidative stress (free radicals).
Zinc (Zn)	Cofactor of many enzymes involved in bone metabolism. Antioxidant role, especially in stabilization of biological membranes. Zinc supplementation reduces bone resorption and enhances bone extracellular matrix mineralization.	Deficiency induces a reduction of the bone mass and a decrease of osteoblasts and osteoclasts numbers in rats. Deficiency can lead to impaired human skeletal growth. Children having a Zn deficiency present a late growing.
Copper (Cu)	Cofactor of many enzymes involved in bone metabolism. Antioxidant role via superoxide dismutase (free radicals elimination).	Copper deficient animals show impairment of secondary bone and cartilage tissues. Free copper ions can generate free radicals.
Manganese (Mn)	Cofactor of many enzymes involved in bone metabolism. Antioxidant role via superoxide dismutase (free radicals elimination).	Deficiency could induce ostarthritis, epiphyseal dysplasia and a disrupted bone mineralization. Excess is neurotoxic.
Selenium (Se)	Antioxidant role via glutathione peroxidases (free radicals elimination).	Long-term deficiency induces growth retardation and impaired bone metabolism.

Materials and methods

Tibet Autonomous Region

10 families were selected in two regions according to three criteria:

- Living in endemic areas
- Including a 3-5 years old child
- Having an older KBD child

Nutritional survey and foods sampling have been done twice: January and May

Nutritional Survey

24-hour food recalls of the 3-5 years old children by the mean of a prospective questionnaire.

Foods sampling

8 foods among the most consumed: barley flour, wheat flour, potato, rice, black tea, yak butter, Chinese cabbage and instant noodles

Foods process

Perishable foods have been dried in a laboratory of the *Tibet Center for Disease Control and Prevention.*

Belgium

Foods mineralization

Microwave-assisted wet process have been performed.

Validation of Analytical Methods

Certified reference materials (CRM) + repeatability

Minerals measurement

Mineralized solutions were measured by several methods:

- Atomic absorption spectrometry (FAAS, ETAAS, HG-AAS and co.Vap.-AAS)
- Atomic emission spectrometry (ICP-AES)
- Molecular absorption spectrometry (UV-Vis)

Foods mineral contents

Evaluation of daily intakes

Comparison with reference tables

Results and discussion

Foods mineral contents

Analytical methods were successfully validated through CRM treated in the same procedure as samples.

Foods mineral contents were expressed in mg/100g of fresh matter (FM) and compared with two reference tables: USDA National Nutrient Database and Souci Fachmann Kraut Food composition tables 1989/90.

Selenium content was too low to be quantified by both methods: HG-AAS and ICP-AES.

The results are as follows:

- Iron content is markedly high in barley flour and black tea. It is also clearly higher than referenced values in wheat flour, potato and butter;
- Selenium content is clearly lower than referenced values in barley flour, wheat flour, rice and Chinese cabbage;
- Black tea (leaves) is rich in several elements (Ca, Fe and Mn). Nevertheless, brewed black tea is drunk so much diluted that mineral supply is really low.

Nutritional survey

The 24-hour food recalls of each child has been listed for January and May. The results of the survey are as follows:

- Tibetan's diet is principally based on cereal products.
- Fruits, meats and dairy products are low consumed.
- Brewed black tea and butter tea are the most common beverages.
- *Chang*, a local made alcohol, is also largely consumed.
- There is a low diversity of recipes.

Evaluation of mineral daily intakes

Daily intakes were estimated combining mineral measurements and nutritional survey results. They were computed via the Kidmenu® software. This software already included Tibetan dish recipes from a previous study of P. de Voghel, 2008. In order to get an approximation of Se intake, LOD and LOQ adapted values were encoded. The results are as follows:

- We confirm a marked deficiency in calcium;
- Ca/P ratio are always low (±0.1);
- Iron and copper intakes are excessive;
- Zinc is the most probably deficient;
- Selenium intakes approximation suggests a deficiency;
- Manganese intakes often exceed toxicity thresholds!

Conclusions and perspectives

This work constitutes an original nutritional study in the Kashin-Beck ethiology context.
The nutritional survey confirmed a monotonous and unbalanced diet.
Most of foods are highly rich in iron while selenium is clearly deficient.
Serious deficiency and excess have been highlighted in young Tibetan children from endemic areas.
Nevertheless, this work presents some limits and restrictions:
Some unmeasured foods were not take into accounts in this study such as *chang*, meat or even water.
They are likely to supply sizeable amounts of minerals though.
Even if executed in two different endemic regions, the observed tendencies cannot be extrapolated to every endemic area. A larger study over a longer term covering both endemic and non-endemic regions is required for definite conclusions to be reached.
Dietary reference intakes can vary with the type of diet and it is important to notice that DRIs tables are established for a standard population with a quite balanced diet. Yet, Tibetans may not be compared to a standard population and they do not have a balanced diet at all. Bioavailability of elements largely influences DRIs. It notably depends on the chemical speciation, on the presence of antinutritional factors (e.g. chelating agents), on the type of diet and on the individual.
The bioavailability of minerals is a critical point that deserves further investigations.