Calcium and Manganese Concentrations in Different Tissues of Clinically Vitamin A Deficient Rats.

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ABSTRACT

Twenty-four male Wistar rats were randomly divided into three groups and fed two types of synthetic diets for 52 days. A(−) group was fed vitamin A deficient diet ad libitum. PF, pair-fed, and A(+) groups were given restricted amount or allowed free access to control diet respectively. In brain, calcium concentration was significantly higher in the A(−) group when compared with the other two groups. In tibia, calcium concentration was significantly lower in the A(−) group compared with the other two. In testis, calcium and manganese concentrations were significantly higher in A(−) group than PF and A(+) groups. These results suggest that vitamin A deficiency affects normal metabolism of calcium and manganese in some tissues of rats.

Key words : Rats, vitamin A deficiency, neurological disorder, calcium, manganese, tissues.

INTRODUCTION

Vitamin A is an unique nutrient and involved in diverse functions such as vision, reproduction, cellular differentiation and immune system regulation. Reports demonstrating interactions between vitamin A and minerals are increasing. It has been reported that vitamin A is involved in the regulation of iron release from liver (Ref. 1) and it can lead to an improvement of iron metabolism (Ref. 2). Accumulation of calcium in brain of central nervous system (CNS) degenerative diseases have been reported (Ref. 3, 4). The essentiality of manganese to mammals was discovered early in 1931 (Ref. 5, 6). Even manganese deficiency have never been reported in free living humans, in a study it has been observed in man in association with a vitamin K deficiency (Ref. 7). Until now, no report demonstrated any effect of vitamin A deficiency on manganese status in any species nor role of any mineral to the genesis of neuro-

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logical disorder in vitamin A deficiency. This study thus was performed to find the effect of vitamin A deficiency on calcium and manganese status and the association between changes of these minerals in tissues and vitamin A-deficiency-related disorders in rats.

MATERIALS AND METHODS

Twenty-four male, 3 weeks old, KBL Wistar rats, weighing about 50g were purchased from Oriental Bio-service, Kyoto, Japan. The rats were divided equally into three groups. They were housed individually in stainless steel cages with raised wire bottom and was placed in a room with controlled temperature (24°－26°C). The rats were fed two types of synthetic diets that was formulated according to AIN 76A (Ref. 8, 9) diet with some modification. Table 1 shows the composition of the diets. A(－) group was allowed free access to vitamin A deficient diet (0 IU vit. A/kg diet). PF and A(＋) groups were fed control diet (4000 IU vit. A/kg diet) but these two groups were given restricted amount (the amount eaten by A(－) group in previous 24 hours) or allowed free access to control diet respectively. Distilled water was allowed to the rats freely. Daily feed intake and weight of individual rats every 5 days and last day were measured. Feed was withheld at the previous night (12 hours) and in the morning of the 54th day, the rats were anaesthetised with sodium pentobarbital and blood was collected from abdominal aorta. Liver, brain, spleen, testis, heart, lung, portion of skeletal muscle and tibia were removed, blotted dry and weighed. Samples were digested by wet ashing in a hot block bath (Model TPB-62, Advantec Toyo Kaisha Ltd., Tokyo, Japan). Calcium and manganese concentrations were determined by a flame atomic absorption spectrometer (Model AA-670, Shimadzu Co., Kyoto, Japan). Reagents and standards used for digestion and analysis were highest grade obtained from Nacalai Tesque Inc. (Kyoto, Japan). Deionized double distilled water was used for analysis.

Data for mineral concentrations and organ weights were analyzed by ANOVA. For group comparison, Bonferroni adjusted probabilities were tested, using $p<0.05$ as the criterion of significance. Systat software package version 5.2 (Ref. 10) was used for the statistical analysis.

RESULTS

No significant weight differences in feed intake were observed among the groups at the begining and until day 10. The aveage body weight of PF group exceeded A(－) group from day 50 and continued. Apart from decreased feed intake and weight gain, classical signs of vitamin A deficiency including lustreless fur, periocular porphyrin deposits and paralysis of limbs at later stages were present in all the rats of A(－) group. However, such signs were absent in rats of PF and A(＋) groups. Regarding organ weights of testis and tibia significant differences were observed between A(－) and PF groups. Atrophy of testis and enlargement of tibial bone were observed in A(－) group (data are not shown). However, no significant difference was observed between PF and A(＋) groups in testis weight. Brain was the only

organ where no significant weight difference was observed among the three groups. Differences in calcium and manganese concentrations are shown graphically in Figures 1 and 2.

In brain, calcium concentration was significantly higher whereas in tibia it was lower in A (−)

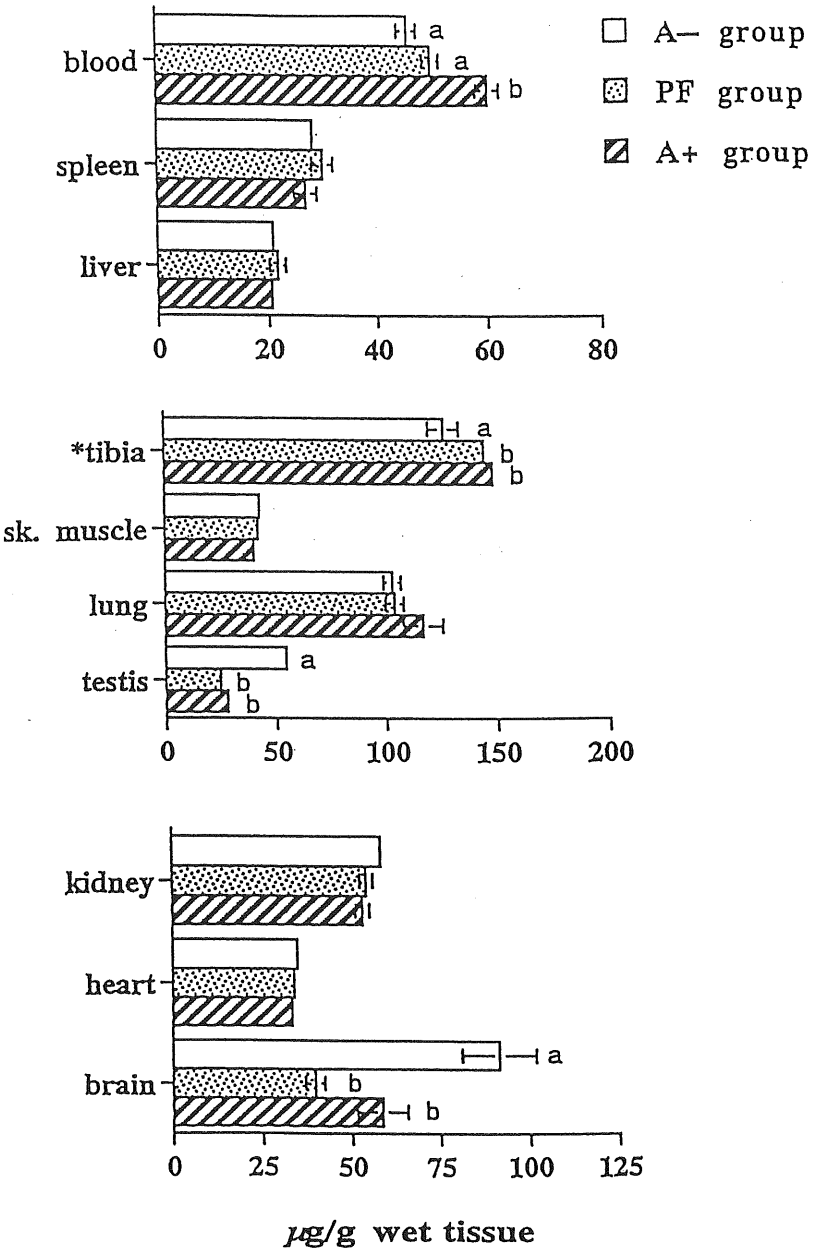

Figure 1. Calcium concentration in different tissues. Bars are mean ± SEM for 8 rats/group. For same tissue bars not sharing same superscript are significantly different at $p<0.05$. *mg/g wet tissue.

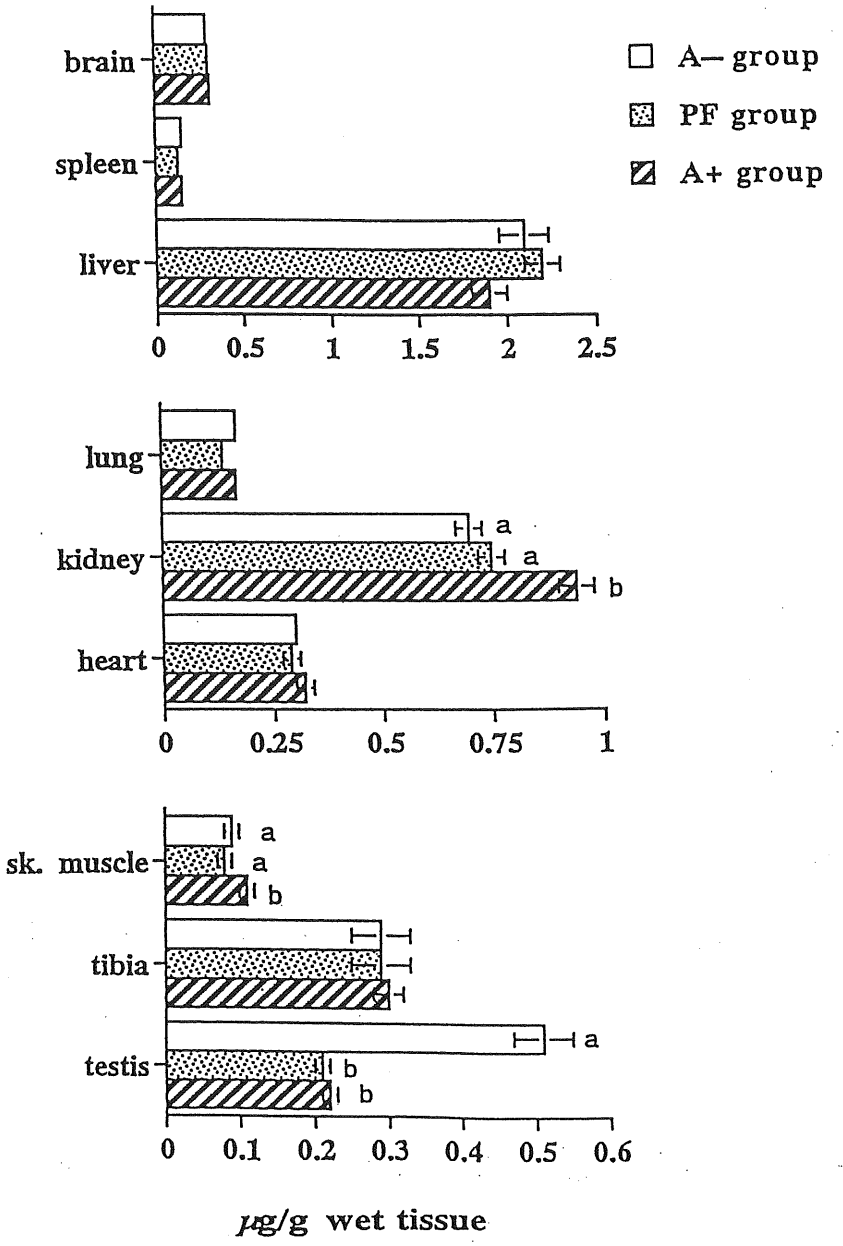

Figure 2. Manganese concentration in different tissues. Bars are mean ± SEM for 8 rats/group. For same tissue bars not sharing same superscript are significantly different at $p<0.05$.

group compared to PF and A(+) groups. In testis, calcium and manganese concentrations were significantly higher in vitamin A deficient rats (A(−) group) than rats of the other two groups.

DISCUSSION

Vitamin A deficiency and zinc deficiency have been reported to cause atrophy of testis (Ref. 11, 12). It has been reported that high concentration of intracellular Ca^{++} is toxic to the cell (Ref. 13). It can be suggested that the atrophy of testis in vitamin A deficient rats (A(−) group) might be the result of excessive amount of calcium in that organ. On the other hand, the accumulation of calcium and manganese in testis of A(−) rats could be a secondary rather than a primary event in the process of degeneration. Lower concentration of calcium in tibia of vitamin A deficient rats was observed in our study. Similar observation has been reported earlier (Ref. 14). The mechanisms involved in bone mineral deregulation in vitamin A deficiency are unclear. Bone mineral concentration in body can be regulated by parathyroid hormone (PTH) in a variety of ways. It has been reported that vitamin A stimulates PTH secretion (Ref. 15). It can be suggested that vitamin A affects normal regulation of bone mineral by interfering PTH secretion. Increased accumulation of calcium in brain of CNS degenerative diseases has been reported (Ref. 3, 4). A definite correlation between calcium, and manganese and other minerals have also been observed in CNS diseases (Ref. 16). Our results suggest that the excessive accumulation of calcium in brain of vitamin A deficient rats might be responsible for the development of neurological disorders in vitamin A deficiency. However, regarding manganese concentration in brain, no significant difference was observed among the three groups.

From our study, definite changes in calcium and manganese metabolism have been observed in some tissues of vitamin A deficient rats. But, the mechanisms involved in such changes are not clear. Further study is needed to clarify the assumptions.

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