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Ascorbic acid
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Calcium

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Bone
Kidney
Brain

Influence of Vitamin C and Magnesium on Calcium, Magnesium and Copper Contents of Guinea Pig Tissues

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Summary: The effects of a high dose of ascorbic acid superimposed on a low magnesium diet were studied for the first time. Young male guinea pigs were fed for six weeks two diets containing 3000 or 600 ppm magnesium: half in each group was supplemented with a daily oral dose of either 3 mg or 100 mg ascorbic acid per 100 g body weight. No treatment effects were found in serum copper and ceruloplasmin, spleen copper, bone calcium, kidney magnesium, and brain calcium and magnesium contents. Both bone copper and brain ascorbic acid contents of the group fed the normal ascorbic acid/low magnesium diet were lower ($p < 0.01$) than the combined means of the other three groups; the high ascorbic acid/low magnesium treatment resulted in normalization of bone copper and brain ascorbic acid levels. Irrespective of ascorbic acid level, the low magnesium diet decreased the bone magnesium and increased the kidney calcium contents ($p < 0.01$); this effect on kidney was nearly doubled by the high ascorbic acid intake ($p < 0.01$). The results indicated that the main effects were due to the magnesium deficit.

Introduction

Considerable information now exists in support of both beneficial and adverse effects of high doses of ascorbic acid on the metabolism of several minerals; among these are iron, copper, and calcium [21]. Species, age, dose and duration of experiments, and target tissue response, all may contribute to variations in results or interpretation. High intakes of vitamin C were reported to adversely affect copper metabolism in early studies of chicks and rats, specially when fed diets low in copper. In young men, high intake of vitamin C also decreased serum ceruloplasmin significantly, but not serum copper [7]. However, guinea pigs [12] and monkeys [13] show slight reductions in ceruloplasmin and copper that are not statistically signifi-

cant [12, 13] when compared to animals receiving normal amounts of the vitamin. Eventual depletion of copper stores in guinea pigs is indicated by significant reductions in liver copper [12, 20] and whole blood copper (20) with daily vitamin C doses of 25 mg/100 g body weight [12] and 250 mg/animal [20].

In early studies of vitamin C and calcium metabolism, mainly animal models that synthesize the vitamin were used. Recently, a reduction in bone density was reported [2] in young (173±23 g) male guinea pigs fed for 43 days a diet containing 8.7% ascorbic acid. This high level of ascorbic acid did not affect bone density of guinea pigs with a starting weight of 236 g [2].

Magnesium deficiency is commonly found in clinical situations. Bone is also adversely affected by its deficiency since, among other functions, magnesium stabilizes the labile soluble calcium phosphate phase of bone. Bone but not soft tissue magnesium declines significantly in young guinea pigs at or below 600 ppm magnesium, but bone calcium is not affected [14]. A magnesium deficit also affects rat bone matrix [11]. An experimentally produced magnesium deficit thus can serve as a model to study the effects of superimposed nutrient excesses; a widely practiced one is vitamin C excess.

This study was designed to investigate the individual and combined effects of high vitamin C and low magnesium intakes on bone and soft tissue calcium, magnesium and copper. The low magnesium diet had one fifth of the recommended level of magnesium for the guinea pig. Serum copper and ceruloplasmin were also measured as two additional parameters of copper status.

Materials and Methods

Twenty four young male, Hartley guinea pigs (Camm, Wayne, NJ) were housed individually in suspended stainless steel cages with wire-bottoms in a room kept at 25–27° with a 12-hour light-dark cycle. They were weighed and provided daily with clean bottles of distilled water and food *ad libitum*. After six days they were matched by weight (230±6 g) and randomly assigned to one of four treatments: normal ascorbic acid with normal magnesium (NA-NM) or low magnesium (NA-LM); high ascorbic acid with normal magnesium (HA-NM) or low magnesium (HA-LM). The normal ascorbic acid was 3.0 mg and the high 100 mg per 100 g body weight per day. Ascorbic acid solutions in 15% dextrose were prepared daily and administered orally in two equal doses, morning and evening, and were adjusted with each 50 g gain in body weight.

The Reid-Briggs pelleted diet [15] was adjusted by ICN Nutritional Biochemicals (Cleveland, OH) to conform to specifications for magnesium and ascorbic acid for this study. Two diets were supplied containing 0.01% vitamin C and 3000 ppm or 600 ppm magnesium; the diets were otherwise nutritionally adequate. On the basis of two-day consumption records, three group were pair fed to the group with the lowest food intake (HA-LM) for the remainder of the experiment.

Analyses. After six weeks the guinea pigs were killed and serum was prepared and frozen at -30°. Brain, spleen and kidneys were quickly removed, weighed and frozen. The left brain was immediately processed at 4° for ascorbic acid analysis. The tibias were cleaned of adhering tissues, weighed and frozen; prior to analysis, these were dried at 60° to constant weight.

Total ascorbic acid was analyzed [17] using, 2,4-dinitrophenylhydrazine and concentrations measured at 540 nm. Ceruloplasmin was assayed as paraphenylenediamine oxidase and results expressed in international units [16].

Mineral concentrations were determined by flame atomic absorption spectroscopy after digestion in concentrated nitric acid and dilution to constant volume. For calcium and magnesium analyses, digests were first diluted with 0.42 M HCl containing 4% TCA (w/v) and 0.6% lanthanum oxide (w/v). Decontamination procedures, methods of additions and other quality control measures were also conducted for mineral analyses.

The data were subjected to two-way analysis of variance and contrast of pooled sample means [3]. Statistically significant results of contrast tests are reported at a probability level of 0.01.

Results

Between group body and organ weights of guinea pigs did not differ at the end of the six-week experiment. Serum copper and ceruloplasmin, and spleen copper (Table I) were not affected by the treatments, although the high vitamin C groups had consistently lower levels of serum copper and ceruloplasmin and higher spleen copper; the differences however were insignificant. The calcium and magnesium contents of the brain (Table I) were also not affected by the treatments. While brain vitamin C levels of the four groups were essentially the same and within acceptable limits (169–208 μg/g), the NA-LM group had a significantly lower mean ($p < 0.01$) than the pooled mean of the other three groups despite comparable tissue levels of magnesium.

The calcium and magnesium contents of tibia were not affected by vitamin C (Table II). Bone magnesium was significantly lower ($p < 0.01$) in the two low magnesium than in the normal magnesium groups. Significant treatment effects of both magnesium and vitamin C were also found for bone copper (Table II). The NA-LM copper level was lower than NA-NM and HA-NM levels ($p < 0.01$), indicating that the low magnesium diet significantly decreased the copper content of bone; at the high vitamin level, this effect was reversed as revealed by similar mean bone copper of NA-NM and HA-LM groups.

Tab. I: Effects of treatments on serum copper and ceruloplasmin, spleen copper, and brain calcium, magnesium and ascorbic acid in young male guinea pigs.

Treatments (6 weeks)	Serum copper μg/ml	Serum ceruloplasmin I.U.	Spleen copper μg/g	Brain calcium μg/g	Brain magnesium μg/g	Brain ascorbic acid μg/g
Normal ascorbic acid						
Normal magnesium	0.82 ± 0.04 (3)[1,2]	55 ± 6 (5)[3]	2.82 ± 0.32 (7)	157 ± 63 (7)	150 ± 3 (7)	206 ± 6 (7)
Low magnesium	0.91 ± 0.06 (3)[2]	66 ± 6 (6)	3.16 ± 0.44 (6)	118 ± 59 (6)	143 ± 2 (6)	169 ± 10 (6)[4]
High ascorbic acid						
Normal magnesium	0.70 ± 0.05 (3)[2]	53 ± 11 (3)[2]	3.31 ± 0.29 (6)	79 ± 13 (6)	147 ± 4 (6)	208 ± 20 (6)
Low Magnesium	0.77 ± 0.05 (3)[2]	49 ± 7 (6)	4.57 ± 0.87 (6)	122 ± 28 (6)	143 ± 2 (6)	208 ± 5 (6)

[1] Mean ± standard error; number of guinea pigs in brackets.
[2] Each N represents pooled serum from two guinea pigs.
[3] International Units = $\mu M \cdot min^{-1} \cdot L^{-1}$.
[4] Significantly lower than the pooled mean of the other three treatments, $p < 0.01$.

Tab. II: Effects of treatments on bone and kidney calcium, magnesium and copper in the male guinea pig.

Treatments (6 weeks)	Bone calcium mg/g	Bone magnesium mg/g	Bone copper µg/g	Kidney calcium µg/g	Kidney magnesium µg/g
Normal ascorbic acid					
Normal magnesium	185 ± 10 (7)[a,1,2]	5.89 ± 0.45 (7)[a]	1.09 ± 0.08 (6)[a]	156 ± 16 (7)[a]	219 ± 13 (7)[a]
Low magnesium	202 ± 5 (6)[a]	3.96 ± 0.40 (4)[b]	0.78 ± 0.15 (4)[b]	488 ± 12 (6)[b]	198 ± 15 (6)[a]
High ascorbic acid					
Normal magnesium	186 ± 7 (5)[a]	5.81 ± 0.33 (6)[a]	1.27 ± 0.11 (6)[a]	156 ± 18 (5)[a]	205 ± 11 (5)[a]
Low magnesium	191 ± 4 (6)[a]	3.55 ± 0.23 (6)[b]	1.04 ± 0.10 (6)[a]	767 ± 24 (6)[b,c]	195 ± 3 (6)[a]

[1] Mean ± standard error. Number of guinea pigs in brackets; bone, as dry weight.
[2] Column means not sharing a common superscript letter are significantly different, $p < 0.01$.

Magnesium contents in kidneys of the four treatment groups (Table II) were not significantly different, although levels in the low magnesium groups were consistently but only slightly lower than in the normal magnesium groups. Calcium levels of the low magnesium groups were higher than the normal magnesium groups ($p < 0.01$). This accretion was aggravated by the high vitamin C intake as revealed by the calcium level of the HA-LM group, which was nearly double ($p < 0.01$) the NA-LM calcium level.

Discussion

Compared to normal controls, the present results and those of others [12, 13] reveal only slight and statistically insignificant reductions in serum ceruloplasmin and copper with high vitamin C treatment. These two parameters are significantly higher in guinea pigs on a vitamin C deficient diet compared to those on a high intake (25 mg/100 g body weight per day) [12]; this pronounced difference may be due to increased glucocorticoids in vitamin C deficiency [9], since these hormones are known to act as stressors for hepatic release of ceruloplasmin. Recently it was also revealed that timing of vitamin C supplementation was critical [5]; aortic lysyl oxidase and ceruloplasmin activities increased when copper-depleted chicks were injected with vitamin C seventy five minutes after a copper dose; these responses were impaired when vitamin C was injected before or with the copper dose. Nevertheless, depletion of hepatic copper is a consistent finding [12, 20] and suggests eventual depletion of copper pools, an effect partially attributed to inhibition of copper absorption by vitamin C [21]. *In vitro,* the vitamin also inhibits binding of hepatic metallothionein and ceruloplasmin to copper [6]. Yet no conclusive evidence exists as to whether *in vivo* redistribution of copper occurs or total body content decreases.

An ambiguous finding is a slight increase in splenic copper with the doubling of splenic vitamin C [20]. The present data (Table I) also show consistent, though in-

significant, increases in splenic copper of high vitamin C treated groups. Our results suggest that, for the duration of the study, delivery of copper to target tissues was not compromised; bone copper declined only in the NA-LM group, signifying the low magnesium was the causal factor.

Brain magnesium and calcium were not affected by the treatments. The vitamin C level in the NA-LM group declined significantly but was within physiologically acceptable limits. Since both magnesium and calcium levels were normal in the NA-LM brain, this finding cannot be easily explained but may be related to the availability of magnesium ions for maximum ATPase activity of brain cell plasma membrane and the energy demand [22] for transport of L-ascorbic acid into brain. Although conservation of magnesium occurs in states of deficit, plasma levels fall and may limit its concentration in cerebrospinal fluid. Under the present conditions, the NA-LM brain may have reached its maximum capacity for active transport of L-ascorbic acid, the normal vitamin level in the HA-LM brain representing additional penetration by passive diffusion.

The low magnesium diet decreased the bone copper content (77% of control) while high vitamin C reversed this effect. This reversal suggests that under destabilizing conditions, e.g. loss of bone matrix due to magnesium deficiency [11], saturating tissue levels of the vitamin *in vivo* [19, 20] enhance anabolic processes in developing bone. This view is supported by several lines of evidence, such as enhancement of aortic lysyl oxidase activity in copper deficient chicks [5], and histologically normal cartilage in surgically induced osteoarthritis in guinea pigs [19]. Studies of articular chondrocytes in culture [10, 18] also provide convincing evidence that supplemental vitamin C enhances several synthetic processes (DNA, collagen, sulfated proteoglycans) and inhibits lytic activity (arylsulfatases, acid phosphatase).

Bone magnesium fell only due to the dietary deficit of magnesium (64% of control). Under the present conditions, neither magnesium nor vitamin C influenced the calcium content of bone. The response of bone to magnesium deficiency has been reliably reproduced in young animal models [8, 14]. Experiments with young guinea pigs [2] comparable in mean age and weight to those used by us (236 and 230 g respectively) also revealed no change in bone density. Our results show 36% of bone magnesium and 23% of bone copper were lost due to the magnesium deficit. Impairment of proteoglycans and collagen in magnesium deficient rats [11] also support the view that bone matrix is degraded. Under such conditions, a higher than normal vitamin C level may be needed for repair.

Kidney calcium accretion in magnesium deficiency without a detectable fall in kidney magnesium (NA-LM) is shown for most soft tissues [8, 14]; this was doubled by the high vitamin C treatment (HA-LM). Two coincident events may be responsible for this accretion, i.e. increased renal calcium phosphate [8] and calcification [4] due to the magnesium deficit, and excess oxalate production [23] due to the high

vitamin C dose. Since kidney calcium of HA-NM and NA-NM groups were the same, a pre-existing magnesium deficit is indicated for further precipitation of calcium with excess oxalate. Such a mechanism is supported by evidence that magnesium oxalate is more soluble than calcium oxalate and reduces the incidence of experimentally produced calcium oxalate stone [1].

In this study, the main experimental effects were due to the limited dietary magnesium. Excess vitamin C superimposed on such a diet reversed the responses in bone and brain but aggravated the calcium accretion in kidney. Additional observations are needed to verify if bone integrity was maintained, and whether or not high vitamin C treatment produces negative copper and calcium balances. Since the guinea pigs were supplemented with vitamin C to simulate a human megavitamin dose, the results on kidney calcium are particularly relevant in cases of concurrent nutrient deficits or imbalances created by disease or therapeutic measures [23]. The results clearly indicate inverse systemic effects and substantiate the view that vitamin C supplementation should be practiced with caution.

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