Effect of Vitamin A Deficiency on Cellular and Intracellular Distribution of Iron, Copper and Zinc

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Introduction

Vitamin A deficiency has consistently been associated with a larger accumulation of iron in tissue[1-4]. The accumulation has been suggested to be due to an alteration in cellular metabolism of iron, rather than due to an excessive absorption[2]. Evidences of biological interaction amongst the chemically related metals of transitional metal series is well established now[5]. The deficiency or excess of some metal creates secondary disorder in the absorption, plasma and tissue levels and also excretion of other metals[6,7]. There is also possibility that they would undergo common metabolic process. High intake of zinc is antagonistic to the utilization of copper and iron[8,9]. Excessive intake of zinc can produce anaemia through reducing the utilization of copper. Moreover, zinc can compete with iron for Fe-binding sites in transferrin. Copper deficiency leads to anaemia which is hypochromic and microcytic in nature, with a concomitant accumulation of iron in liver and spleen[10,11]. An appropriate ratio amongst the metals is thus necessary for their proper utilization. Any sort of imbalance in the utilization of copper and zinc may eventually produce secondary effect in the utilization of iron. Both copper and zinc have already been claimed to be related to the metabolism of vitamin A; zinc being related in its capacity to mobilize vitamin A from the liver[12,13], and copper being related in its capacity to facilitate the storage of vitamin A in the liver[21]. Moreover, retinol and copper are reported to behave similarly in being stored preferentially in liver and being carried in blood plasma in combination with similar but different alpha2-globulin[32]. Thus the deficiency or excess of one is likely to produce change in the metabolism of other. Likewise there exists the possibility that vitamin A deficiency may affect the metabolism of copper and zinc which, in turn, may have secondary effect on the balance of iron. In view of this assumption, the present experiment was undertaken where both cellular and intracellular distribution of iron, copper and zinc was determined in vitamin A deficient rats and compared with those of control.

Materials and Methods

Fifteen post weanling rats of Long-Evans strain weighing 75g were made deficient in vitamin A by feeding them vitamin A deficient basal diet (Table 1). Another group of fifteen rats were fed control diet containing vitamin A. Control diet was prepared by supplementing the dificient diet with

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vitamin A (8000 I.U./kg diet). All rats were provided with water ad libitum. This dietary regime was continued for 7-weeks, till the appearance of vitamin A deficiency amongst the rats fed vitamin A deficient diet, as was indicated by weight plateau. Amount of iron, copper and zinc in the diet was kept as usual. Supply of vitamin A was the only difference between the control and deficient diets. After depletion, rats were sacrificed under mild chloroform anesthesia by decapitation. The organs and serum samples were preserved in deep freeze at -18°C until vitamin A iron, copper and zinc were analysed. Hemoglobin was estimated by collecting blood from tail-tip before sacrifice. All glasswares used in preservation and mineral analysis were soaked in 1N 1:1 HCl:HNO_3 for at least 24 hr and rinsed ten times with glass distilled water.

Analytical methods-Subcellular fractions of liver were separated by differential centrifugation after homogenisation of the tissue[14]. Estimation of Fe, Cu and Zn in whole organs as well as in subcellular fractions of liver was accomplished by atomic absorption spectrophotometer after subjecting the samples to wet digestion with 1:4 concentrated $HClO_4$.HNO_3 and proper dilution. Serum samples were diluted appropriately (10 times) and aspirated directly into atomic absorption nebulizer for the estimation of Cu and Zn in serum[15]. Serum iron was determined colorimetrically by using orthophenanthroline as chromogenic reagent[16]. Liver vitamin A was estimated colorimetrically by using TCA[17]. Hemoglobin was determined by cyanmethemoglobin method using Sigma commercial Kit[18]. Serum vitamin A was determined by HPLC[19]. Difference between the values of control and deficient animals was determined by Student's t-test[20].

Results

As depicted in Table 2, rats fed vitamin A deficient diet showed significant depletion ($P<0.001$) of serum vitamin A and liver vitamin A, indicating a vitamin A deficient state. Vitamin A deficient rats also showed significantly ($P<0.001$) lower values for body weight and hemoglobin as compared to the control.

Effect of vitamin A deficiency on tissue content of Fe, Cu and Zn is described in Table 3.

Effect on iron-Rats fed vitamin A deficient diet showed a larger deposition of iron in liver and spleen, but a lower concentration of iron in kidney and serum. While iron content of brain remained unaffected by vitamin A. deficiency.

Effect on copper-Vitamin A deficient rats showed significantly higher concentration of copper in serum. While copper content of liver, spleen, kidney and brain was found to remain unaffected by vitamin A deficiency.

Effect on zinc-Vitamin A deficient rats were found to have larger store of zinc in liver, but lower level of zinc in serum. While concentration of zinc in spleen, kidney and brain did not alter significantly as a result of vitamin A deficiency.

Effect of Vitamin A deficiency on subcellular distribution of iron,

copper and zinc in liver is depicted in Table 4; and their corresponding percent distribution amongst sub-cellular compartments is described in Table 5.

Effect on subcellular distribution of iron-Subcellular distribution of iron in liver was found to be altered as a result of vitamin A deficiency. Greater accumulation of iron was found to be attached to debris, cytosol and large granules of vitamin A deficient liver, as compared to the control. Moreover, vitamin A deficiency was found to cause a decrease of iron in microsomal fraction of liver. In both control and deficient liver, pattern of iron distribution was found to be in the order of - cytosol> debris> large granules> microsomes. Percent distribution of iron was found to be increased in cytosol, but decreased in microsomal fraction as a result of vitamin A deficiency.

Effect on subcellular distribution of copper-Subcellular distribution ol copper in liver was affected by vitamin A deficiency with its lower concentration being attached to cytosol and concomitant higher concentration being attached to large-granules. While concentration in debris and microsomes was found to remain unaffected. Pattern of copper-distribution in vitamin A deficient liver was found to be in the order of-debris, large-granules, cytosol> microsomes, as compared to-cytosol >debris>large-granules> microsomes in control. Proportion of copper was found to be increased in large-granules, but decreased in cytosol.

Effect on subcellular distribution of zinc-Vitamin A deficiency was found to alter the subcellular distribution of zinc with its higher concentration being attached to debris, cytosol and microsomal fraction; zinc concentration in large-granules remaining unaffected. Intracellular distribution of zinc in deficient liver was found to be in the order of-cytosol> debris> large- granules> microsomes as compared to-debris, cytosol>large-granules>microsomes in control. Proportion of zinc was found to be decreased in large-granules as compared to the control.

1. Skimmed milk powder was washed with hot ethyl alcohol.

2. Vitamin mixture was premixed with bulk of rice flour to provide the required amounts of vitamins per kg diet in 6 percent combination. Vitamin A was excluded in vitamin mixture.

3. Control diet was prepared by adding vitamin A (8000I.U./kg diet) in the form of retinylacetate to the deficient diet.

Table 1. *Composition of vitamin A deficient basal diet*[28]

Ingredients	Percentage
Rice flour	50
Skimmed milk powder	35
Soybean oil	5
Vitamin mixture[29]	6
Salt mixture[30]	4

Table 2. *Weight, vitamin A status and hemoglobin of rats fed vitamin A deficient and control diets.*

Measures	Groups	
	Control	Deficient
Body weight (g)	270.35 ± 8.12[a]	230.33 ± 7.25[b]
Hemoglobin (g/dl)	15.05 ± 1.06[a]	11.25 ± 0.65[b]
Liver vitamin A (μg/g)	145.25 ± 10.15[a]	8.33 ± 1.02[b]
Serum vitamin A (μg/dl)	60.25 ± 5.66[a]	14.75 ± 1.12[b]

Values are mean ± S.D. (n = 15). Values within same line sharing different superscripts are significantly (P< 0.001) different.

Table 3. *Effect of vitamin A deficiency on iron, zinc and copper content of different tissues of rats.*

Elements	Tissue content (μg/gwet. wt.)				Serum (μg/dl)
	Liver	Spleen	Kidney	Brain	
Iron:					
Control	169.56 ± 9.48[a]	249.26 ± 12.10[a]	52.09 ± 4.06[a]	23.28 ± 3.71[a]	145.35 ± 10.50[a]
Deficient	265.35 ± 16.40[b]	369.87 ± 15.87[b]	35.15 ± 2.20[b]	21.72 ± 2.20[a]	102.75 ± 7.95[b]
Copper:					
Control	13.81 ± 1.27[a]	4.80 ± 0.29[a]	7.25 ± 9.50[a]	5.86 ± 0.40[a]	115.80 ± 5.90[a]
Deficient	14.15 ± 1.07[a]	5.10 ± 0.28[a]	7.14 ± 0.35[a]	5.77 ± 0.49[a]	162.45 ± 4.35[b]
Zinc:					
Control	29.34 ± 2.60[a]	23.34 ± 2.15[a]	18.22 ± 1.31[a]	16.72 ±1.08[a]	120.35 ± 4.70[a]
Deficient	38.09 ± 2.35[b]	22.50 ± 1.25[a]	19.10 ± 1.96[a]	15.57 ± 1.05[a]	80.25 ± 3.50[b]

Values are mean ± S.D. (n =15). For each element, values within the same column bearing different superscripts are significantly different (P< 0.001).

Table 4. *Effect of vitamin A deficiency on subcellular distribution of iron, copper and zinc in the liver of rats*

Elements	Total content of elements (µg) in subcellular fractions/g tissue.				Mean total (µg/g wet.wt.)
	Debris	Large granules	Microsomes	Cytosol	
Iron:					
Control	52.86 ± 5.48[a]	30.83 ± 2.16[a]	17.35 ± 1.02[a]	64.01 ± 5.41[a]	165.05
Deficient	84.02 ± 6.70[b]	52.80 ± 3.08[b]	11.21 ± 0.81[b]	126.57 ± 7.99[b]	274.60
Copper:					
Control	3.80 ± 0.24[a]	2.73 ± 0.17[a]	1.60 ± 0.11[a]	5.99 ± 0.18[a]	14.12
Deficient	3.99 ± 0.11[a]	3.91 ± 0.22[b]	1.75 ± 0.27[a]	3.75 ± 0.25[b]	13.40
Zinc:					
Control	9.83 ± 0.82[a]	6.38 ± 0.31[a]	3.90 ± 0.18[a]	10.25 ±1.15[a]	30.36
Deficient	12.90 ± 1.03[b]	6.74 ± 0.19[a]	5.69 ± 0.17[b]	15.36 ± 1.05[b]	40.69

Values depicted in the table are mean ± S.D. (n = 15). For each element, values within same column bearing different superscripts are significantly different (P< 0.001). Mean total is calculated by summing the mean values of individual fractions. Debris-(nuclei, plasma membrane, unbroken cell); large granules-(mitochondria and lysosome); microsomes-(endoplasmic reticulum, golgi apparatus and ribosomes).

Table 5. *Effect of vitamin A deficiency on percent distribution of iron, copper and zinc within subcellular compartments of liver of rats.*

Elements	% Distribution in				Total
	Debris	Large granules	Microsomes	Cytosol	
Iron :					
Control	32.03	18.67	10.52	38.78	100
Deficient	30.60	19.23	4.08	46.09	100
Copper :					
Control	26.92	19.33	11.33	42.42	100
Deficient	29.78	29.18	13.06	27.98	100
Zinc:					
Control	32.37	21.03	12.84	33.76	100
Deficient	31.70	16.57	13.98	37.75	100

Percent distribution was calculated from their mean values. Debris-(nuclei, plasma membrane, unbroken cell); large granules-(mitochondria and lysosomes); microsomes-(endoplasmic reticulum, golgi apparatus, ribosomes).

Discussion

Lower level of hemoglobin and serum iron in vitamin A deficient rats is in agreement with similar findings in previous studies of vitamin A deficiency[1-4]. Accumulation of iron in liver and spleen is also consistent with similar finding in vitamin A deficient rats by Mejia et al[1,2], and Hodges et al[3]. Iron accumulation in liver and spleen has been attributed to an immobilization of iron from the stores in absence of vitamin A.

Lower level of iron in kidney of vitamin A deficient rats might be related to some metabolic specificity of kidney towards iron or vitamin A. Kidney differs from other organs in its capacity to degrade RBP (retinol binding protein) after its life span. Retinol released from RBP breakdown products in kidney is reutilised in the body. Due to this capacity, kidney is continuously flushed with a supply of retinol from RBP[31]. Thus it is most likely that during vitamin A deficiency when liver vitamin A- storage is diminished, kidney-iron is raided off for hematopoiesis with the help of retinol whatever is available from RBP breakdown products. Depression of hematopoiesis along with an accumulation of iron in the liver and spleen would suggest an impaired utilization of iron in absence of vitamin A.

In the present study, concentration of copper in serum increased following the deprivation of vitamin A. Since keratinization is known to need the participation of copper[21], this higher level of copper in serum might be as a response to increased demand for copper needed for increased keratinization during vitamin A deficiency. An inverse relationship between vitamin A and copper in serum has also been reported in copper toxicosis and in copper deficiency[21]. Moore et al[21] in their experiment with copper poisoned sheep demonstrated an inverse correlation between retinol and copper in blood, but no such relationship was observed in liver. It was suggested that in copper toxicosis, the capacity of liver to mobilize retinol to circulation is disrupted due to the fact that excessive demands for the carrier of excessive copper in serum are met at the expense of failure in the production of RBP, resulting in lower mobilization of retinol to blood. Owen et al as cited by Moore et al[21], reported an inverse relationship between copper and retinol concentration in the blood of kids made deprived of copper. It was suggested that an abnormal accumulation of retinol in blood occured through the inefficient absorption of retinol by the copper deficient liver and pointed to the claims that copper may facilitate the storage of retinol in liver. Though copper deficiency is known to lead to iron accumulation in the liver,[10,11] vitamin A deficient liver with larger accumulation of iron has been found to have normal level of copper. Thus iron accumulation in present condition is not through the reduction of copper in the system.

In our present experiment, vitamin A deficient rats had a greater storage of zinc in the liver with a decrease of zinc in serum. Functional interaction between vitamin A and zinc has been reported in previous studies[12,13].

Zinc deficiency is known to depress serum retinol by disrupting the hepatic synthesis of RBP, the carrier protein for retinol. In the present study, the situation is different with a larger store of zinc in liver and lower level of zinc in serum of vitamin A deficient rats. Larger store of zinc is in agreement with a similar finding of a larger accumulation of zinc in the liver of vitamin A depleted chicken by Sifri et al[22]. Berzin et al[23] reported a negative balance of zinc in vitamin A deficient chicken. Moreover, they were able to isolate a vitamin A dependent Zn-binding protein (ZnBP) from the ileal mucosa of vitamin A repleted chicks which was absent in vitamin A deficient ones. It was suggested that ZnBP is involved in the binding of zinc in the ileal mucosa of chick for its proper absorption. Likewise lower level of zinc in the serum of rats in present study might be related to an impaired absorption of zinc due to vitamin A deficiency. At the same time, larger store of zinc in liver might be the result of a decreased utilization of zinc which was supposed to be used up for the mobilization of vitamin A in normal condition. Thus it is most probable that zinc has been spared by its decreased turnover in absence of adequate vitamin A in liver. Alternatively vitamin A might be needed for a proper mobilization of zinc from liver; and during vitamin A deficiency an impaired mobilization might have resulted in its lower concentration in serum along with a larger accumulation in liver.

Subcellular distribution of iron, copper and zinc in liver was markedly altered by vitamin A deficiency. Highest amount of copper and iron in the liver of control rats was found to be attached to cytosol with the next higher concentration being associated with debris. However zinc in debris and cytosol was found in comparable amounts. In the liver of normal rat, highest concentration of iron, zinc and copper is generally associated with cytosol, with their next higher concentration generally being located in debris[24,25]. Highest concentration of the metals in cytosol appears to be related to the fact that depository proteins for these metals are synthesized in cytosol.

Thus, accumulation of iron in the liver of vitamin A deficient rats occured primarily in cytosol, debris and large-granules, but with a simultaneous shift of iron from microsomes. Bigger share of debric-iron might be located at plasmamembrane and unbroken cell mass, since nuclei is not known to contain considerable amount of iron. Lysosomal iron load is one of the important characteristic of general iron loading. In present study, larger accumulation of iron in large-granules may account for an accumulation in lysosome, since mitochondria is known to be protected from excess iron during general loading except due to abnormality in haem synthesis itself[26]. Due to exogenous loading, iron accumulation increases proportionately in all cell compertments, but endogenous iron loading gives rise to different situation with disproportionate loading, for example mitochondrial loading during sideroblastic anaemia and lysosomal accumulation associated with the aging pigment in neurons[27]. In view

of this concept, it may be inferred that hepatic iron accumulation under the influence of vitamin A deficiency is an endogenous process, since the accumulation is disproportionate in nature. The imbalance in the matabolism of Fe, Cu and Zn is further documented by their decompartmentalization amongst subcellular particles. This decompartmentalization may be due to some alteration in the synthesis as well as in the distribution of their storage proteins. Though functional interpretation of such alteration in subcellular distribution is not possible in the present context, this eventually may affect their proper utilization. Moreover, the iron accumulation during vitamin A deficiency does not seem to develop from an antagonism from Cu and Zn, though it was assumed that iron accumulation might be a disorder secondary to the action of vitamin A deficiency on the metabolism of Cu and Zn.

Summary

Cellular and intracellular distribution of iron, copper and zinc was determined in vitamin A deficient rats. Vitamin A deficiency caused a larger accumulation of iron in liver and spleen, and a decrease of iron in kidney and serum. Iron concentration in brain remained unaffected. Level of serum copper increased as a result of vitamin A deficiency. Whilst concentration of copper in liver, spleen, kidney and brain remained unaffected. Vitamin A deficiency also caused a larger store of zinc in liver, with a concomitant fall of zinc in serum; whilst zinc concentration in spleen, kidney and brain remained unaffected. Subcellular distribution of iron, copper and zinc in liver was altered by vitamin A deficiency. Iron content in debris, cytosol and large-granules increased, but decreased in microsomal fraction as a result of vitamin A deficiency. In both control and vitamin A deficient liver, iron distributed itself in the pattern of-cytosol> debris> large-granules> microsomes. Proportion of iron decreased in microsomes, but increased in cytosol. Likewise, copper content decreased in cytosol, but increased in large-granules as a result of vitamin A deficiency. In vitamin A deficient liver, copper distributed itself in the order of- debris, cytosol, large-granules> microsomes, as compared to cytosol> debris> large-granules> microsomes in control. Proportion of copper increased in large-granules and microsomes, but decreased in cytosol. Subcellular distribution of zinc was affected with its higher concentration being attached to debris, cytosol and microsomes. In vitamin A deficient liver, intracellular distribution of zinc was found to be in the order of-cytosol>debris>larg-granules> microsomes, as compared to-cytosol, debris> large-granules> microsomes in control. Proportion of zinc decreased in large-granules. Iron accumulation during vitamin A deficiency does not appear to develop due to the interaction of iron with copper or zinc.

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