

## Erythrocyte, lipid peroxidation and antioxidant enzymes in hypervitaminotic A rats and their modification by dietary protein

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Rats fed excess vitamin A showed decreased body weight gain and protein efficiency ratio. In rats fed low protein vitamin A level increased in liver but with an associated decrease in plasma. These changes were reversed in high protein fed state. The amount of protein in diet had little effect on haemoglobin level in erythrocyte, but excess vitamin A in diet significantly decreased haemoglobin level in erythrocyte. Lipid peroxidation (LP) increased in rats fed low protein and decreased in high protein fed rats. Rats fed high protein and excess vitamin A showed minimum level of LP. Result showed that high protein in diet increased the levels of antioxidant enzymes, catalase and superoxide dismutase (SOD) and that excess vitamin A supplementation functions synergistically with high protein in diet to increase antioxidant enzymes level.

Vitamin A is transported by protein, the retinol-binding protein (RBP) in plasma. RBP level increases/decreases following excess/low intake of protein in diet respectively<sup>1</sup>. Therefore low protein intake decreases the transport of vitamin A from liver to plasma and accumulation of vitamin A in liver becomes more and its corresponding level in plasma becomes less whereas in high protein fed state plasma vitamin A level increases due to enhanced transport while in liver it decreases<sup>1,2</sup>. Thus protein intake influences transport of vitamin A. Vitamin A has been shown to cause lysis of erythrocytes *in vitro*<sup>3,4</sup> and can inhibit tissue lipid peroxidation (LP)<sup>5,6</sup>. Vitamin A supplementation increases the activity of antioxidant enzymes, catalase and SOD<sup>7</sup>. Effects of normal and excess dietary vitamin A on RBC haemoglobin, tissue LP and antioxidant enzymes in varying dietary protein conditions have been estimated in this investigation to find out whether low and excess dietary protein intake modifies the effect of vitamin A on the above parameters in rats.

### Materials and Methods

Four months old male albino rats of Wistar strain weighing 140-160 g were obtained from Central

Animal House of the University Department of Experimental Medicine. They were individually housed in polypropylene cages in a room at  $25^{\circ} \pm 2^{\circ}\text{C}$  with 12: 12 hr L:D cycle. The modified diets were fed to the rats<sup>8</sup>. They were given water *ad libitum*.

**Chemicals**—All the chemicals used were of analytical grade and all the vitamins used were of I.P. grade. Analytical grade all trans retinol was obtained from Nicholas Piramol (India) Limited, Mumbai, as gift. Glass distilled water was used for all the analyses.

**Experimentation**—The local Institutional Animal Ethics Committee clearance was obtained for the investigation. Initially 36 rats were equally divided into 6 groups (Group I-VI). Groups I, II and III were fed normal level of vitamin A (4000 IU/kg diet) containing diets whereas groups IV, V and VI were fed high level of vitamin A (40,000 IU/kg diet) containing diets. Group I and group IV diets contained normal level of casein (18%), group II and group V diets contained low level of casein (6%) and groups III and VI diets contained high level of casein (24%). All the administered diets (Groups I-VI) were made isocaloric by keeping the total weights of protein (casein) and carbohydrate (rice starch) same and also by adding same amount of lipid (groundnut oil). Each rat was given 20 g diet per day for 30 days. Rats of Groups I-VI consumed 2760, 2910, 2550, 2580, 2760 and 2400 g of their respective diets during 30 days. At the end of

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30<sup>th</sup> day all the rats were fasted overnight for 12 hr and sacrificed on the morning of 31<sup>st</sup> day by decapitation using ether anaesthesia.

The composition of 1kg standard diet and experimental diets is as follows :

Group I (control) diet and group IV diet : casein 180 g, rice starch 670 g, salt mixture 40 g, groundnut oil 100 g and vitamin mixture 10 g.

Group II diet and group V diet : low protein diets contained 60 g casein and 790 g rice starch; other constituents were same as control diet.

Group III diet and group VI diet: high protein diet contained 240 g casein and 610 g rice starch; other constituents were same as control diet.

Powdered retinyl palmitate tablet (50,000 IU) was mixed with the diet at 4000 IU/kg and 40,000 IU/kg as per the protocol above.

**Collection of blood**—Blood was collected from experimental rats by cutting carotid artery into heparinised tubes containing 1mg sodium salt of heparin/5ml of blood.

The protein content in plasma/liver homogenate was estimated as per Lowry *et al.*<sup>9</sup>.

Vitamin A from liver was extracted by the method of Ames *et al.*<sup>10</sup> and subsequently estimated from the

chloroform phase as per Carr Price<sup>11</sup>. Vitamin A in plasma was estimated by the method of Jalal *et al.*<sup>12</sup> using high performance liquid chromatographic technique (HPLC)<sup>2</sup>.

Haemoglobin content in haemolysate<sup>13</sup>, LP in liver<sup>14</sup>, plasma<sup>15</sup> and erythrocyte<sup>16</sup>, the activities of catalase<sup>17</sup> and SOD<sup>18</sup> were measured.

Results expressed are the mean  $\pm$  SE of 6 rats in each group. Student's *t*-test was used to determine the statistical significance between control and test groups. Significance level was fixed at 0.05.

## Results and Discussion

Protein deficiency significantly decreased the body weight (group II; Table 1). This may be due to deficiency of amino acids and decrease of activity of hepatic and probably other tissue microsomal oxidases<sup>19</sup>. Excessive intake of dietary protein can be deleterious due to increased catabolic reactions and unbalanced interactions among amino acids<sup>20</sup> (group III; Table 1). The lower body weight gain in rats fed excess vitamin A may be due to toxic effect of vitamin A<sup>21,22</sup>. Protein efficiency ratio (PER) was found to be decreased as protein content in diet increased (groups II & III; Table 1). This may be due to increased

Table 1—Effect of dietary protein on body weight change, protein efficiency ratio (PER) and vitamin A levels in plasma and liver in normal and hypervitaminotic A rats

[Values are mean  $\pm$  SE of 6 rats. Figures in parentheses are % increase (+) or decrease (–) over control (Group I)]

| Groups | Parameters                              |                                |                                          |                                            |
|--------|-----------------------------------------|--------------------------------|------------------------------------------|--------------------------------------------|
|        | Body weight change (g)                  | Protein efficiency ratio (PER) | Vitamin A level in plasma ( $\mu$ g/dl)  | Vitamin A level in liver ( $\mu$ g/g)      |
| I      | 109.83 $\pm$ 3.14                       | 1.06                           | 25.44 $\pm$ 7.85                         | 180.86 $\pm$ 16.12                         |
| II     | 82.17 $\pm$ 3.16 <sup>a</sup><br>(–25%) | 1.49<br>(+41%)                 | 17.80 $\pm$ 4.44 <sup>NS</sup><br>(–30%) | 385.64 $\pm$ 17.93 <sup>a</sup><br>(+113%) |
| III    | 91.00 $\pm$ 3.85 <sup>b</sup><br>(–17%) | 0.76<br>(–28%)                 | 35.21 $\pm$ 6.37 <sup>NS</sup><br>(+38%) | 56.52 $\pm$ 5.28 <sup>a</sup><br>(–69%)    |
| IV     | 85.00 $\pm$ 3.18 <sup>a</sup><br>(–23%) | 0.88<br>(–17%)                 | 32.20 $\pm$ 3.36 <sup>NS</sup><br>(+27%) | 234.66 $\pm$ 7.12 <sup>a</sup><br>(+30%)   |
| V      | 70.83 $\pm$ 3.10 <sup>a</sup><br>(–36%) | 1.35<br>(+27%)                 | 19.58 $\pm$ 3.11 <sup>NS</sup><br>(–23%) | 575.32 $\pm$ 13.29 <sup>a</sup><br>(+218%) |
| VI     | 78.00 $\pm$ 3.04 <sup>a</sup><br>(–29%) | 0.69<br>(–35%)                 | 39.47 $\pm$ 4.85 <sup>NS</sup><br>(+55%) | 133.75 $\pm$ 5.12 <sup>a</sup><br>(–26%)   |

P values : <sup>a</sup> < 0.001, <sup>b</sup> < 0.01

NS : Non Significant

Group I : Normal protein + Normal vitamin A

Group II : Low protein + Normal vitamin A

Group III : High protein + Normal vitamin A

Group IV : Normal protein + Excess vitamin A

Group V : Low protein + Excess vitamin A

Group VI : High protein + Excess vitamin A

amount of fecal and urinary nitrogen excretion<sup>23</sup>. Decreased PER in rats fed excess vitamin A compared to normal vitamin A fed rats could be due to less body weight gain (group I vs group IV; group II vs group V & group III vs group VI; Table 1).

Low protein in diet decreases the synthesis of RBP which in turn affects the synthesis of vitamin A from liver to plasma resulting accumulation of vitamin A in liver and corresponding level in plasma becomes low<sup>2</sup>

(group II; Table 1). The situation was reversed when the protein intake was more. The condition became worse when excess vitamin A was supplemented in diet (group II; Table 1).

No significant change was observed in haemoglobin levels among groups I, II and III (Table 2). This indicates that quantity of protein in diet may have little effect on haemoglobin content in RBC. Haemoglobin levels were significantly decreased in all the

Table 2—Effect of dietary protein on RBC haemoglobin and liver, plasma and RBC lipid peroxidation (TBARS) level in normal and hypervitaminotic A rats

[Values are mean  $\pm$  SE of 6 rats. Figures in parentheses are % increase (+) or decrease (–) over control (Group I)]

| Groups | Parameters                              |                                                                 |                                                       |                                                          |
|--------|-----------------------------------------|-----------------------------------------------------------------|-------------------------------------------------------|----------------------------------------------------------|
|        | Haemoglobin level in RBC (g/dl)         | Lipid peroxidation (TBARS) level in liver (n mol/mg of protein) | Lipid peroxidation (TBARS) level in plasma (n mol/dl) | Lipid peroxidation (TBARS) level in RBC (p mol/mg of Hb) |
| I      | 12.65 $\pm$ 0.35                        | 1.17 $\pm$ 0.06                                                 | 201.74 $\pm$ 8.60                                     | 4.11 $\pm$ 0.12                                          |
| II     | 11.59 $\pm$ 0.42 <sup>NS</sup><br>(–8%) | 1.41 $\pm$ 0.09 <sup>NS</sup><br>(+21%)                         | 266.51 $\pm$ 5.62 <sup>a</sup><br>(+32%)              | 4.21 $\pm$ 0.11 <sup>NS</sup><br>(+2%)                   |
| III    | 13.05 $\pm$ 0.37 <sup>NS</sup><br>(+3%) | 0.94 $\pm$ 0.05 <sup>c</sup><br>(–20%)                          | 163.49 $\pm$ 14.25 <sup>NS</sup><br>(–19%)            | 2.96 $\pm$ 0.09 <sup>a</sup><br>(–28%)                   |
| IV     | 9.95 $\pm$ 0.41 <sup>b</sup><br>(–21%)  | 1.07 $\pm$ 0.13 <sup>NS</sup><br>(–9%)                          | 173.08 $\pm$ 16.13 <sup>NS</sup><br>(–14%)            | 3.26 $\pm$ 0.11 <sup>a</sup><br>(–21%)                   |
| V      | 9.32 $\pm$ 0.28 <sup>a</sup><br>(–26%)  | 1.27 $\pm$ 0.12 <sup>NS</sup><br>(+9%)                          | 241.94 $\pm$ 9.31 <sup>NS</sup><br>(+20%)             | 3.93 $\pm$ 0.19 <sup>NS</sup><br>(+4%)                   |
| VI     | 9.45 $\pm$ 0.27 <sup>a</sup><br>(–25%)  | 0.80 $\pm$ 0.04 <sup>a</sup><br>(–32%)                          | 144.26 $\pm$ 16.22 <sup>c</sup><br>(–28%)             | 2.65 $\pm$ 0.12 <sup>a</sup><br>(–35%)                   |

P values : <sup>a</sup> < 0.001, <sup>b</sup> < 0.01, <sup>c</sup> < 0.05

NS : Non Significant

Details of Groups I – VI are same as in Table 1.

Table 3 — Effect of dietary protein on liver and RBC catalase and SOD levels in normal and hypervitaminotic A rats

[Values are mean  $\pm$  SE of 6 rats. Figures in parentheses are % increase (+) or decrease (–) over control (Group I)]

| Groups | Parameters                                |                                         |                                        |                                        |
|--------|-------------------------------------------|-----------------------------------------|----------------------------------------|----------------------------------------|
|        | Catalase level in liver (U/mg of protein) | Catalase level in RBC (U/mg of protein) | SOD level in liver (U/mg of protein)   | SOD level in RBC (U/mg of protein)     |
| I      | 1.89 $\pm$ 0.19                           | 2.00 $\pm$ 0.08                         | 4.69 $\pm$ 0.47                        | 2.80 $\pm$ 0.08                        |
| II     | 1.74 $\pm$ 0.15 <sup>NS</sup><br>(–8%)    | 1.67 $\pm$ 0.07 <sup>c</sup><br>(–17%)  | 4.52 $\pm$ 0.05 <sup>NS</sup><br>(–4%) | 2.59 $\pm$ 0.09 <sup>NS</sup><br>(–6%) |
| III    | 2.11 $\pm$ 0.13 <sup>NS</sup><br>(+12%)   | 2.23 $\pm$ 0.06 <sup>NS</sup><br>(+12%) | 4.80 $\pm$ 0.07 <sup>NS</sup><br>(+2%) | 3.04 $\pm$ 0.11 <sup>NS</sup><br>(+9%) |
| IV     | 2.23 $\pm$ 0.09 <sup>NS</sup><br>(+18%)   | 2.44 $\pm$ 0.09 <sup>b</sup><br>(+22%)  | 4.94 $\pm$ 0.09 <sup>NS</sup><br>(+5%) | 3.17 $\pm$ 0.08 <sup>c</sup><br>(+13%) |
| V      | 1.96 $\pm$ 0.09 <sup>NS</sup><br>(+4%)    | 1.97 $\pm$ 0.09 <sup>NS</sup><br>(–2%)  | 4.62 $\pm$ 0.05 <sup>NS</sup><br>(–1%) | 2.92 $\pm$ 0.09 <sup>NS</sup><br>(+4%) |
| VI     | 2.40 $\pm$ 0.07 <sup>c</sup><br>(+27%)    | 2.86 $\pm$ 0.01 <sup>a</sup><br>(+43%)  | 5.03 $\pm$ 0.06 <sup>NS</sup><br>(+7%) | 3.48 $\pm$ 0.08 <sup>a</sup><br>(+24%) |

U (Catalase)  $\rightarrow$   $\mu$  mol of H<sub>2</sub>O<sub>2</sub> utilised per minute

U (SOD)  $\rightarrow$  Enzyme concentration required to inhibit 50% of chromogen production

P values : <sup>a</sup> < 0.001, <sup>b</sup> < 0.01, <sup>c</sup> < 0.02

NS : Non Significant

Details of Groups I – VI are same as in Table 1

groups supplemented with excess vitamin A compared to their counterparts of normal vitamin A supplemented groups. Vitamin A may have increased the susceptibility to oxidative lysis of RBC and thereby reduced the level of haemoglobin in RBC. RBC is susceptible to oxidative damage due to (i) as oxygen transporter it is exposed to high oxygen tension (ii) the RBC membrane is rich in polyunsaturated fatty acids (PUFAs) which are readily converted to unstable lipid radicals or (iii) the cell is rich in iron, a potent catalyst for production of reactive oxygen species<sup>24</sup>.

Liver, plasma and RBC LP were increased in group II and decreased in group III as compared to group I (Table 3). Inadequate intake of dietary protein may be responsible for enhanced LP. The degree of protein deficiency affects the extent of the enhancement of tissue LP in rats<sup>25</sup>. LP of the above tissues were inhibited by feeding of excess vitamin A in the present study. Decreased level of LP may be indicative of increased vitamin A transport which subsequently helps to quench the free radical and decreases LP. *In vivo* inhibitory capacity of retinol could be suggestive of its ability to penetrate into endoplasmic reticulum and protects the phospholipids therein from free radical attack<sup>26</sup>.

Protein deficiency decreased the levels of catalase and SOD. Protein deficiency depresses the levels of antioxidative enzymes<sup>25</sup>. Increased levels of catalase and SOD in excess vitamin A supplemented groups indicate that excess vitamin A increases the activities of scavenging enzymes<sup>7</sup>.

It can be summarised that in hypervitaminotic-A rats body weight, haemoglobin and LP decrease, while antioxidant enzymes, catalase and SOD increase. Results also show that excess vitamin A supplementation in diet functions synergistically with high protein diet to reduce LP and to increase antioxidant enzymes.

## References

- 1 Ingelbleek Y, Van Den Schrick H G, De Naeyer & De Visscher M Albumin, transferring and thyroxin – binding prealbumin/retinal—binding protein (TBPA/ RBP) complex in assessment of nutrition, *Clin Chim Acta*, 63 (1975) 61.
- 2 Karar P K, Manavalan R & Rajagopal G, Effect of dietary protein on vitamin A levels in plasma and liver in hypervitaminotic A rats, *Indian J Exp Biol*, 40 (2002) 355.
- 3 Kartha V N R & Krishnamurthy S, Vitamin A induced hemolysis and lipid peroxidation. Effect of vitamin A E, D and antioxidants, *Curr Sci*, 42 (1973) 225.
- 4 Callani D, Costa M & Billitteri A, Retinol induced hemolysis and metabolic conditions of erythrocytes, *Boll Soc Ital Biol Super*, 54 (1978) 435.
- 5 Kartha V N R & Krishnamurthy S, Antioxidant function of vitamin A, *Int J Nutr Vit Res*, 47 (1977) 394.
- 6 Kartha V N R & Krishnamurthy S, Effects of vitamins, antioxidants and sulfhydryl compounds on *in vitro* rat brain lipid peroxidation, *Int J Nutr Vit Res*, 48 (1978) 38.
- 7 Helen A & Vijayagammal P L, Effect of vitamin A supplementation on cigarette smoke induced lipid peroxidation, *Vet Hum Toxicol*, 39 (1997) 18.
- 8 Pugalendi K V & Ramakrishnan S, Intestinal cholesterol synthesis and mobilisation to blood in sucrose – feeding and alcohol – intake, *Indian J Exp Biol*, 38 (2000) 762.
- 9 Lowry O H, Rosebrough N J, Farr A L & Randall R J, Protein measurement with Folin reagent, *J Biol Chem*, 193 (1951) 265.
- 10 Ames S R, Risley H A & Harris P L, Simplified procedure for extraction and determination of vitamin A in liver, *Anal Chem*, 26 (1954) 1378.
- 11 Carr T H & Price E A, Color reactions attributed to vitamin A, *Biochem J*, 20 (1926) 497.
- 12 Jalal F, Nesheim M C, Agus Z, Sanzur D & Habicht J P, Serum retinol concentrations in children are affected by food sources of  $\beta$ -carotene, fat intake and anthelmintic drug treatment, *Am J Clin Nutr*, 68 (1998) 623.
- 13 Drabkin D L & Austin J M, Spectrophotometric studies, spectrophotometric constants for common haemoglobin derivatives in human, dog and rabbit blood, *J Biol Chem*, 98 (1932) 719.
- 14 Ohkawa H, Ohisi N & Yagi K, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, *Anal Biochem*, 95 (1979) 351.
- 15 Yagi, Lipid peroxides and human disease, *Chem Phys Lipids*, 45 (1987) 337.
- 16 Cynamon H A, Isenberg J N & Nguycan C H, Erythrocyte malondialdehyde release *in vitro*, a functional measure of vitamin E status, *Clin Chim Acta*, 51 (1985) 169.
- 17 Sinha K A, Colorimetric assay of catalase, *Anal Biochem*, 47 (1972) 389.
- 18 Kakkar P, Das B & Viswanathan P N, A modified spectrophotometric assay of superoxide dismutase, *Indian J Biochem Biophys*, 21 (1984) 130.
- 19 Campbell T C & Hayes J R, Role of nutrition in the drug – metabolizing enzymesystem, *Pharmacol Rev*, 26 (1975) 171.
- 20 Adrienne E, Nutrition, in *The Laboratory rat*, Vol. 1, edited by Henry J B, Lindsey J R & Steven H W (Academic Press, New York) 1979, 130.
- 21 Deluca L M, in *Hand book of lipid research*, Vol.2, edited by H F Deluca (Plenum Press, New York and London) 1978, 1.
- 22 Bauernfeind J C, *The safe use of vitamin A: A report of the International Vitamin A Consultative Group*, (The Nutrition Foundation, Washington D C) 1980.
- 23 Bunce G E & King K W, Amino acid retention and balance in the young rat fed varying levels of casein, *J Nutr*, 98 (1969) 168.
- 24 Hebbel R P, Erythrocyte antioxidants and membrane vulnerability, *J Lab Clin Med*, 107 (1986) 401.
- 25 Huang C J & FWV M L, Degree of protein deficiency affects the extent of the depression of the antioxidative enzyme activities and the enhancement of tissue lipid peroxidation in rats, *J Nutr*, 123 (1993) 803.
- 26 Thomas G, Sanjeev Kumar P, Jayanthibai N & Krishnamurthy S, Antioxidant and haemolytic functions of retinol, *Nutr Report Int*, 24 (1981) 1205.