Effect of dietary protein on vitamin A levels in plasma and liver of hypervitaminotic-A rats

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Level of vitamin A increased in plasma and liver in hypervitaminotic A albino rats fed normal quantity of protein in diet. In low protein fed state vitamin A level in liver increased due to accumulation of vitamin A and lack of carrier protein with an associated decrease of plasma vitamin A. In high protein fed rats the level of vitamin A in plasma increased due to enhanced transport while in liver it decreased. The results indicate that for normal transport of vitamin A adequate plasma protein level is essential.

Transport of vitamin A in plasma requires retinol binding protein. Dietary intake of more amount of protein increases its level both in liver and plasma and low protein diet decreases its level. A dietary intake of excess vitamin A requires more amount of protein in diet for its own transport to plasma, thereby protein level decreases in liver but increases in plasma. The present work carried out in albino rats, aims at understanding the role of dietary protein levels when normal amounts of the vitamin A or excess is fed. Indian Council of Medical Research (ICMR) has recommended booster dose of vitamin A to combat vitamin A deficiency in children. But in low socio-economic groups of population, protein malnutrition commonly co-exists with vitamin A deficiency. In the present investigation rats have been fed low protein, normal protein and high protein with excess amount of vitamin A. Their plasma and liver vitamin A levels have been measured to determine the role of dietary protein in control of vitamin A levels.

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° ± 2°C with 12:12 hr L:D cycle. The diet fed to the rats was modified suitably from the diet used by Pugalendi KV and Ramakrishnan S and water ad libitum.

**Chemicals**—All the chemicals used in this experiment were of analytical grade. For HPLC analysis methanol, hexane and isopropanol of HPLC grade were used. All the vitamins used were of I.P grade. Analytical grade all trans retinol and retinyl acetate were obtained from Nicholas Piramal (India) Limited, Mumbai. Glass distilled water was used for all the analysis whereas HPLC grade water was used for HPLC analysis.

**Experimentation**—The local Institutional Animal Ethics Committee clearance was obtained for this investigation. Initially 24 rats weighing 140-160g were equally divided into 4 groups (Group I-IV). Group I was fed normal vitamin A (4000 IU/kg diet) containing diet whereas group II, III and IV were fed high level of vitamin A (40,000 IU/kg diet) containing diets. Group I and Group II diets contained normal level of casein (18%), group III diet contained low level of casein (6%) whereas group IV diet contained high level of casein (24%). All the administered diets (Group I-IV) were made isocaloric by keeping the total weights of protein (casein) and carbohydrate (rice starch) same and also by adding same amount of lipid (ground nut oil) in all the diets. Each rat received 20 g diet per day for 30 days. At the end of 30th day all the rats were fasted overnight for 12 hr and sacrificed on the morning of 31st day by decapitation using ether anaesthesia.
The composition of 1 kg standard diet and different other diets are as follows:

Group I (control diet) and Group II diet: casein 180 g, rice starch 670 g, salt mixture 40 g, ground nut oil 100 g and vitamin mixture 10 g.

Group III diet: low protein diet contained 60 g casein and 790 g of rice starch; other constituents were same as control diet.

Group IV 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 4,000 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 1 mg sodium salt of heparin/5 ml of blood.

The method of Folch et al.2 was adopted for lipid extraction. The protein content in plasma/liver homogenate was estimated as per Lowry et al.3

Determination of Vitamin A—Vitamin A from liver was extracted by the method of Ames et al.4 and subsequently estimated from the chloroform phase by the method of Carr Price5. Vitamin A in plasma was estimated by high performance liquid chromatographic (HPLC) technique6. Shimadzu (Japan) high performance LC6A liquid chromatograph equipped with SPD-6A-UV spectrophotometric detector was used. The separation was performed using Lichrosphere RP-18 (125 x 4 mm i.d, 5 μ particle, Merck) and the guard columns were Lichrosphere RP-18 (4 x 4 mm – i.d, 5μ). All trans retinol was used as standard.

Determination of tissue and plasma lipids—Total cholesterol7, triacylglycerol8, phospholipids9, HDL-cholesterol10 and LDL-cholesterol11 were estimated as per standard procedures.

Results are expressed as the mean ± SE of six rats in each group. Student’s t-test was used to determine the statistical significance between control and test group. Significance levels were fixed at 0.05 level.

The results are presented in Tables 1 and 2.

Body weight changes—The less weight gain of all the excess vitamin A fed rats may be due to toxic effect of vitamin A12,13. This toxic effect was found to be maximum in group III where protein intake was low but vitamin A intake was high.

Total protein content in liver and plasma—Excess vitamin A may require excess amount of retinol binding protein (RBP) for its own transport to plasma as a result of which less RBP and also less total protein were present in liver and more in plasma. In the retinol deficient state, the secretion of RBP from the liver was blocked, resulting in the accumulation of an enlarged pool of apo-RBP in liver and a concomitant decline in serum RBP level14,15. Conversely, the repletion of retinol into retinol deficient rats stimulated rapid secretion of RBP from the liver into plasma16. Release of RBP from existing pool in the liver rather than newly synthesised protein when the vitamin A intake is high is reported17.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
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<tbody>
<tr>
<td>Total protein content in liver (mg/g)</td>
<td>142.11 ± 6.88</td>
<td>127.64 ± 8.60 N.S (−10)</td>
<td>29.81±1.54* (−79)</td>
<td>172.83±8.20* (22)</td>
</tr>
<tr>
<td>Total protein content in plasma (g/dl)</td>
<td>6.36 ± 0.15</td>
<td>6.99 ± 0.13* (+10)</td>
<td>3.85 ± 0.13* (-39)</td>
<td>7.80 ± 0.15* (+23)</td>
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<tr>
<td>Vitamin A content in liver (μg/g)</td>
<td>180.86 ± 16.12</td>
<td>234.66 ± 7.12* (+30)</td>
<td>575.32±13.29* (+218)</td>
<td>133.75±5.12* (-26)</td>
</tr>
<tr>
<td>Vitamin A content in plasma (μg/dl)</td>
<td>25.44 ± 7.85</td>
<td>32.20 ± 3.36 N.S. (+26)</td>
<td>19.58±3.11 N.S. (-23)</td>
<td>39.47±4.85 N.S. (+55)</td>
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P values: * <0.001; ** <0.05;  NS <0.02
Gr. I = Normal protein + normal vitamin A; Gr. II = Normal protein + excess vitamin A; Gr. III = Low protein + excess vitamin A; Gr. IV = High protein + excess vitamin A.
Table 2—Effect of dietary protein on liver and plasma lipids in hypervitaminotic-A rats  
(Values are mean ± SE of 6 rats. Figures in parentheses are % increase (+) or decrease (-) over control (Gr. 1))

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<tr>
<td>Total cholesterol in liver (mg/g)</td>
<td>2.75 ± 0.19</td>
<td>2.86 ± 0.17&lt;sup&gt;NS&lt;/sup&gt; (+4)</td>
<td>3.38 ± 0.08&lt;sup&gt;b&lt;/sup&gt; (+23)</td>
<td>2.68 ± 0.14&lt;sup&gt;b&lt;/sup&gt; (+3)</td>
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<tr>
<td>Triacylglycerol in liver (mg/g)</td>
<td>2.48 ± 0.05</td>
<td>2.61 ± 0.05&lt;sup&gt;NS&lt;/sup&gt; (+5)</td>
<td>3.37 ± 0.18&lt;sup&gt;d&lt;/sup&gt; (+36)</td>
<td>2.39 ± 0.05&lt;sup&gt;NS&lt;/sup&gt; (-4)</td>
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<tr>
<td>Phospholipids in liver (mg/g)</td>
<td>6.04 ± 0.62</td>
<td>7.24 ± 0.38&lt;sup&gt;NS&lt;/sup&gt; (+20)</td>
<td>9.21 ± 0.64&lt;sup&gt;d&lt;/sup&gt; (+52)</td>
<td>5.97 ± 0.40&lt;sup&gt;NS&lt;/sup&gt; (-1)</td>
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<tr>
<td>Total cholesterol in plasma (mg/dl)</td>
<td>61.96 ± 2.24</td>
<td>68.06 ± 2.37&lt;sup&gt;NS&lt;/sup&gt; (+10)</td>
<td>55.84 ± 0.47&lt;sup&gt;b&lt;/sup&gt; (+10)</td>
<td>87.70 ± 3.62&lt;sup&gt;a&lt;/sup&gt; (+42)</td>
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<tr>
<td>Triacylglycerol in plasma (mg/dl)</td>
<td>84.78 ± 2.71</td>
<td>91.34 ± 3.15&lt;sup&gt;NS&lt;/sup&gt; (+8)</td>
<td>82.42 ± 3.94&lt;sup&gt;NS&lt;/sup&gt; (-3)</td>
<td>107.91 ± 11.21&lt;sup&gt;NS&lt;/sup&gt; (+27)</td>
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<tr>
<td>Phospholipids in plasma (mg/dl)</td>
<td>101.74 ± 4.93</td>
<td>112.22 ± 5.35&lt;sup&gt;NS&lt;/sup&gt; (+10)</td>
<td>96.53 ± 4.80&lt;sup&gt;d&lt;/sup&gt; (-5)</td>
<td>125.86 ± 4.52&lt;sup&gt;d&lt;/sup&gt; (+24)</td>
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<tr>
<td>HDL cholesterol in plasma (mg/dl)</td>
<td>24.22 ± 1.16</td>
<td>22.75 ± 1.21&lt;sup&gt;d&lt;/sup&gt; (+6)</td>
<td>19.40 ± 1.37&lt;sup&gt;b&lt;/sup&gt; (-20)</td>
<td>24.43 ± 1.27&lt;sup&gt;d&lt;/sup&gt; (+1)</td>
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<tr>
<td>LDL cholesterol in plasma (mg/dl)</td>
<td>20.79 ± 1.57</td>
<td>27.04 ± 1.21&lt;sup&gt;c&lt;/sup&gt; (+30)</td>
<td>20.62 ± 1.30&lt;sup&gt;NS&lt;/sup&gt; (-1)</td>
<td>46.01 ± 2.08&lt;sup&gt;a&lt;/sup&gt; (+121)</td>
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P values: a<0.001; b<0.05; c<0.02; d<0.01; NS non-significant
Details of groups I-IV are same as in Table 1.

Vitamin A content in liver and protein—Low protein in diet decreases the synthesis of RBP which in turn affects the transport of vitamin A from liver to plasma<sup>18</sup>. Accumulation of vitamin A in liver becomes more and its corresponding level in plasma becomes low. The condition becomes worse when high amount of vitamin A is supplemented along with low dietary protein intake due to excess accumulation of vitamin A in liver (group III; Table 1). The situation was reversed when the protein intake was more (group IV). Thus it is clear that both low protein and high protein in diet have negative impact on the metabolism of vitamin A. Protein nutrition must be taken into consideration when vitamin A is administered to patients. It was reported<sup>19</sup> that metabolism of vitamin A was highest with intake of a high protein diet and thus metabolism of vitamin A was modulated by protein.

Lipid content in liver and plasma—Insufficient dietary protein decreases the synthesis of apoprotein by liver, as a result lipoprotein synthesis also decreases<sup>20</sup>. Since lipoprotein transports cholesterol, triacylglycerol and phospholipids, protein deficiency affects the transport of the above lipids for want of transport protein and lipid remains in the liver (Table 2). This in turn decreases the concentration of the above lipids in plasma. Excess intake of vitamin A requires additional amount of RBP. It appears that there was a diversion of amino acids in liver for the synthesis of transport protein of vitamin A and as a result less amount of apoproteins were available for transport of lipids. This leads to deposition of more cholesterol, triacylglycerol and phospholipids in liver (group III; Table 2).

In the present study, in all the excess vitamin A supplemented groups, LDLc/HDLc ratios were more than normal vitamin A fed groups. The increase of LDLc/HDLc ratio in excess vitamin A fed groups was due to increase of LDL cholesterol in excess vitamin A fed groups than in normal vitamin A fed groups (Table 2). Probably, LDL uptake in liver could have been reduced due to high cholesterol content in hepatic cells. This would suggest that high cholesterol content of hepatic tissue would reduce the expression of LDL receptors and resulting in the elevation of LDL cholesterol.

References