SERUM RETINOL LEVEL OF HUMAN SUBJECTS FED CAROTENE RICH DIET

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ABSTRACT
The study was conducted in 2005 at Department of Agricultural Chemistry, NWFP Agricultural University, Peshawar, Pakistan. The objective was to observe the apparent effect of \( \beta \)-carotene on serum retinol level of 10 volunteers who were fed spinach as a source of \( \beta \)-carotene. The vitamin A contents before and after feeding the test diet was determined by HPLC (High Performance Liquid Chromatography). The data revealed that average vitamin A content of the sera varied from 10.7 to 35.0 \( \mu \)g/dl before feeding the test diet. After feeding the test diet, the average serum retinol content was increased from 20.07 to 37.1 \( \mu \)g/dl in majority of the subjects. Considerable variation was noted among the subjects in response to the same dose of carotenous diet. In four subjects, there was marked increase in the sera retinol content, while in five subjects, though there was an increase but it was not pronounced. One of the subjects showed no response to conversion of beta carotene to vitamin A. It was worth mentioning that no subject complained of the adverse effects of the diet. The subjects remained healthy during the experimental period and afterwards. It was therefore, concluded that carotene had no side effects on human health, rather the diet was protective and healthy as a source of vitamin-A.

INTRODUCTION
Vitamin A, or retinol, is an essential micronutrient for humans and other mammalian species since it cannot be synthesized within the body. Deficiency of the vitamin results in adverse effects on growth, reproduction and resistance to infection. Vitamin A deficiency (VAD) refers to any state in which the vitamin A status is subnormal. It can be presumed to occur when the habitual intake of the total vitamin A is markedly below the recommended dietary intake (WHO, 1976). The most important manifestation of severe vitamin A deficiency is xerophthalmia, which eventually leads to irreversible blindness in one or both eyes. VAD is still an important micronutrient deficiency problem in many developing countries including Pakistan, afflicting large number of pre-school children. It is often associated with protein-energy malnutrition, parasitic infestation and diarrhoeal disease. International Union of Nutritional Sciences (IUNS, 1982) recommends that vitamin A level for adult men and women should be in the range of 15-25 \( \mu \)g/dl, with an increased level of 20-30 \( \mu \)g/dl during pregnancy and lactation.

Measurement of the serum or plasma vitamin A level remains the most important biochemical indicator of vitamin A status of a population (Underwood and Lasgot, 1984). Since vitamin A is stored in high concentrations in the body almost entirely in liver, plasma level does not closely reflect its level in the body as a whole. The plasma concentration reflects the body status only under two very different circumstances; when the body stores have been critically depleted, and when the liver has become saturated with vitamin A.

A basic tool in carotenoid and retinoid research and development activities is the content of these two groups of compounds in foods. In recent years, there has been particular emphasis on understanding the types and concentrations of various carotenoids in foods. It was felt that previously reported values of vitamin A activity in food composition tables may have been unrealistic, since available methodologies were not standardized and the reliable methods were non existent. (Britton, 1995, Simpson and Chichester, 1981, Baurenfeind, 1972). With the advent of precision equipments, \( \beta \)-carotene, the most potent precursor of vitamin A was determined using HPLC. Further the effect of beta carotene was observed on serum retinol level. This was used for optimization of the method and also for studying the effect of beta carotene on serum retinol level in humans.

MATERIALS AND METHODS
The study was conducted in 2005, at Department of Agricultural Chemistry, NWFP Agricultural University, Peshawar, Pakistan. Ten healthy volunteer human subjects from lower and upper middle class community were selected between ages 25-40. The serum was assayed before providing the test diet and after taking the test diet (spinach = 240g/ day) for a week. The test diet consisted of spinach cooked in water with addition of small amounts of water, salt, vegetable oil and spices. The cooking time and tenderness of leaves was the same as usually
practiced in homes. The diet was fed to the subjects along with usual bread and water. The subjects were allowed their daily routine activities. They were fed the prescribed diet once a day (at lunch time) for a week. At the end of the feeding period, blood samples from each subject were taken on 8th day at 8 am before taking breakfast.

The blood samples were centrifuged for 10 min at 12,000 rpm. After centrifugation, the supernatant (serum) was collected by Pasteur pipette and taken into Appendorf tubes. All the samples were stored at freezing temperature until analyzed.

The extraction and determination of vitamin A from blood serum was performed by the method of Islam et al. (2004) slightly modified to optimize the assay. Two hundred µl of sera were taken into an Appendorf tube and 100µl ethanol was added. After thorough mixing, 400µl n-hexane was also added. The mixture was again mixed well with Vortex mixer for 1 min, and then centrifuged at 12,000 rpm for 10 min. The upper solvent layer was collected and evaporated to dryness. The dried residue was dissolved in 100 µl ethanol. Each sample extract of vitamin A (20µl) was injected into HPLC (High Performance Liquid Chromatograph, Perkin Elmer, Model LC 250 Isocratic Pump) when the injector was in “load” mode. HPLC was equilibrated by running mobile phase (methanol + water = 95:5, v/v respectively) at the rate of 1.5ml per min with initial pressure of the column kept at 1390-1500 PSI. Wave length was fixed at 326 nm. After 10 min the run was stopped.

A distinctive sharp peak of vitamin A appeared in the chromatogram after 3.3 min (Rt = 3.3). The vitamin A content of serum was calculated from the standard curve, which was prepared as follows:

Standard of vitamin A (1g in sealed vial) was obtained from Merck. Stock solution was prepared by taking 10mg of vitamin A in 100ml n-hexane. The concentration of stock solution was equal to 100ppm. The stock solution was diluted to different known concentrations by the formula \( C_1 V_1 = C_2 V_2 \) where 20, 40 and 60ppm dilutions were obtained in 5 ml of each n-hexane solution. Each standard solution was injected into HPLC maintaining the as described earlier for serum Assay. The peak was plotted against concentration to construct the curve. The curve was used to find the vitamin A content of the sera.

### RESULTS AND DISCUSSION

In order to assess the conversion of beta carotene to vitamin A in human body, sera of 10 adult human subjects, who were fed the richest carotene vegetable, were assayed. The data shown in Table-I reveals that average vitamin A content of sera varied from 10.7 to 35.0µg/dl in blood before feeding the test diet. After feeding the test diet, the serum retinol content was increased in majority of the subjects. Considerable variation was noted among the subjects in response to the same dose of carotene derived from test diet. In four subjects (C, D, H and I), there was marked increase in the sera retinol content, while in five subjects (A, E, F, G and J), though slight there was an increasing trend of blood serum retinol. One of the subjects (B) showed no response to the conversion of beta carotene to vitamin A (Table I).

The four subjects (C, D, H and I) that had shown maximum increase in serum retinol might be due to high intake of dietary fat because they were selected from the upper middle class and their diet consisted of vegetables along with dietary fatty substances. The more the fat the high will be the conversion rate (Nuray and Torsten et al. 2005). The subjects (A, B, E, F, G and J) which showed no or little increase were selected from lower class community. Their daily diet was not consisting of enough dietary fats although the uptake of vegetables was high. Another reason may be that the retinol is stored in the liver. When it is needed in other parts of the body, the liver attaches vitamin A to a specific protein named retinol binding protein (RBP), which in turn is attached to pre-albumin, both of which are synthesized by liver. If the liver is unable to generate these proteins, the vitamin A will not be lost from the body, but metabolites of vitamin A are excreted by way of bile into the feces (Cunha et al. 2001). The persons who eat more fatty diet with retinol source, their retinol demand is lesser from plant source and hence apparent level of their blood serum retinol would be lower (Khan et al. 1989).

The results of the present study showed that carotene conversion to retinol is dependent on the physiology of individual as well as the past history because various enzymes are involved in the process of carotene to retinol conversion and these enzymes might be varied from individual to individual (Williams et al. 1984). The conversion might also depend on the need of retinol to the body, the greater the need, the
greater the conversion and so would be the level of serum retinol.

It was worth mentioning that no subject complained of the adverse effects of the diet. The subjects remained healthy during the experimental period and afterwards. This indicated that carotene had no side effects on human health, and that, as a source of vitamin A the diet was protective and healthy.

The data of this study are in fair agreement with those of Cynotech and Konatene, (1988) who conducted similar type of experiment. They also recorded considerable inter individual variations in blood retinol levels after administering the oral dose of carotene-rich diet in the form of powdered algae (approx 135mg).

The results were also supported by the work of Tang et al (2005) who supplied fresh raw spinach (300g) to one group of seven human subjects and non-fresh raw spinach (300g) to the second group of seven human subjects for 10 days. They observed that mean retinol content of the first group was increased from 23 to 42.3 µg/dl as compared to the mean retinol content of the second group, which increased from 15.5 to 28.3 µg/dl. They concluded that spinach could provide significant amounts of vitamin A depending upon the bioavailability of plant carotenoids and their efficiency of conversion to vitamin-A.

The work of Jensson et al. (1986) who used carrot as a source of alpha and beta carotene in 17 adult human subjects for a week and then examined their serum retinol content also supported the observations of the present study.

CONCLUSION AND RECOMMENDATION
It is concluded from the present study that High Performance Liquid Chromatography (HPLC) is a rapid, efficient and sensitive technique for serum retinol analysis. Also carotene had no side effects on human health, rather the diet was protective and healthy as a source of vitamin A. The vegetables are fairly good sources of micro nutrient (vitamin A) and hence they can alleviate vitamin A deficiency in masses of the country. Vegetables are cost effective and can substitute the animal food sources, which are expensive and beyond the purchasing power of low income groups of population, especially in rural areas. Therefore the vegetables should be included regularly in their diets.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Before (µg/dl)</th>
<th>After (µg/dl)</th>
<th>*%diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10.7</td>
<td>19.4</td>
<td>81.31</td>
</tr>
<tr>
<td>B</td>
<td>14.1</td>
<td>16.2</td>
<td>14.89</td>
</tr>
<tr>
<td>C</td>
<td>22.1</td>
<td>40.5</td>
<td>83.26</td>
</tr>
<tr>
<td>D</td>
<td>35.0</td>
<td>55.4</td>
<td>58.29</td>
</tr>
<tr>
<td>E</td>
<td>17.0</td>
<td>30.7</td>
<td>80.59</td>
</tr>
<tr>
<td>F</td>
<td>32.8</td>
<td>38.5</td>
<td>17.38</td>
</tr>
<tr>
<td>G</td>
<td>10.8</td>
<td>25.6</td>
<td>137.04</td>
</tr>
<tr>
<td>H</td>
<td>22.9</td>
<td>68.7</td>
<td>200.00</td>
</tr>
<tr>
<td>I</td>
<td>15.0</td>
<td>40.3</td>
<td>168.67</td>
</tr>
<tr>
<td>J</td>
<td>20.3</td>
<td>35.6</td>
<td>75.37</td>
</tr>
<tr>
<td>Mean</td>
<td>20.07</td>
<td>37.1</td>
<td>91.68</td>
</tr>
</tbody>
</table>

T test 2.425
Probability 0.042

*Formula for % difference (Bi-Ai) x 100/Ai
Where,
A= reading before feeding test diet;
B= reading after feeding test diet
i= A, B……J i.e. individuals
REFERENCES


