S-13-3 Comparative Aspects of Human Vitamin K Metabolism and Nutriture

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I. INTRODUCTION
Several aspects of human vitamin K metabolism and nutriture invite comparison. Of central importance is the role of phylloquinone (vitamin K1) versus menaquinones (vitamins K2), and their relative contribution in meeting the needs for vitamin K, not only for the synthesis of the hepatic coagulation factors but also for the less well understood vitamin K-dependent carboxylation reactions in other tissues such as bone. Largely unanswered questions surround the comparative intermediary metabolism and transport of phylloquinone and menaquinones and their comparative intracellular distribution and in vivo activity.

Perhaps the most perplexing question is the nutritional origin of tissue menaquinones (MKs) and more specifically: does the enormous microfloral population of the large intestine provide an important nutritional source of vitamin K or are MKs largely of dietary origin?

Another area of possible comparison is health versus disease. For example an important question is how various disease states may influence the metabolism and hence the needs for vitamin K or our perception of these needs. Some results pertaining to the above questions are addressed below.

II. DIET VERSUS MICROFLORA AS A SOURCE OF MENAQUINONES
Using modern chromatographic techniques it has been now established that the majority of human hepatic stores of vitamin K consist of long chain menaquinones [1,2]. Although the human utilization of menaquinones has been questioned [3], the problem of their origin remains. Some observations from our laboratory may be pertinent to this question.

Firstly food analyses by high performance liquid chromatography (HPLC) have shown that certain foods such as dairy produce (cheese, yogurt, milk) contain significant and measurable amounts of menaquinones. Of the above foods cheeses had the highest MK levels; the vitamers detected in order of magnitude being normally MK-9, MK-8, MK-7, MK-10, MK-6 and MK-5. A peak corresponding to MK-4 was also detected but its identity requires further confirmation. The concentrations of MK-9 and MK-8 in cheeses were quite substantial and usually around 20 micrograms/100g and 10 micrograms/100g respectively. It is of interest however that this profile does not match adult human livers where MK-7 or MK-10 or both are generally present in greater amounts than MK-9. Even longer chain MKs such as MK-11 and
MK-12 are often present in human liver but these were not evident in any foods analysed except for the livers of other animals which are eaten only rarely by most people.

Thus although MKs in food could provide a useful source of vitamin K it seems unlikely that the entire liver content and spectrum of forms derives from the diet alone. Textbook dogma of course suggest that MKs are derived from the enteric bacteria and it usually assumed that this process occurs from the colon. There is no doubt that this region contains the largest population of MK-producing bacteria but on physiological grounds [4] significant absorption of MKs from the colon seems doubtful. It is possible that a more favourable absorption occurs from the terminal ileum but the presence of menaquinones in this region has not been demonstrated previously. Therefore in collaboration with Dr R.V. Kries we studied the concentrations and possible bioavailability of K vitamins in ileal juice obtained from six volunteers who were undergoing routine endoscopy. The results showed that the members of the series MK-6 to MK-10 were readily detectable in most subjects, although the concentrations in individual subjects were as variable as those seen in human liver samples. In 5 out of 6 subjects MK-10 was the predominant form at concentrations ranging from 8-81 ng/ml while the remaining subject had large concentrations of MK-7 and MK-8 (64 and 91 ng/ml respectively). This variable pattern of menaquinones in ileal juice fits in well with the variability of hepatic stores but again the bioavailability of MKs from ileal juice seems questionable since virtually all the MKs could be removed from solution by simple centrifugation or by passing through a 0.2 micron filter. This suggests that the MKs in ileal juice are still membrane bound and not in a micellar form which would favour the absorption process.

III. PHYLLOQUINONE VERSUS MENAQUINONES - METABOLIC CONSIDERATIONS

There is a marked difference in lipophilicity between phylloquinone and some of the apparently important human sources of menaquinones such as MKs 7-10. Such molecular differences are now known to influence their tissue distribution. For instance, although hepatic concentrations of MKs 7 and 10 almost always exceed that of phylloquinone, the latter vitamer is the predominant circulating vitamer in plasma. In fact most laboratories only report the measurement of phylloquinone since the detection of menaquinones is difficult. In our experience, using a method based on HPLC with redox mode electrochemical detection, MK-7 is readily detectable in most plasma samples. In a preliminary study the mean concentration of MK-7 in 11 normal subjects was 0.29 ng/ml compared to 0.51 ng/ml for K1 [5].

In a more recent study in 20 haemodialysis patients (in collaboration with Dr J. Saupe) although the mean total concentrations of K1 and MK-7 were raised (due to secondary hyperlipidaemia in some but not all of the patients, see section IV below) the ratio of MK-7 to K1 was 0.7, this being very similar to the ratio in normals of 0.6 [5]. In contrast, the mean concentration of MK-8 in haemodialysis patients (0.33 ng/ml) was much lower than that reported [5] for normal subjects (0.54 ng/ml). This difference may be due to methodological problems in the assay of MK-8. Also in the haemodialysis group greater proportions of MK-7 and MK-8 (22% and 24% respectively) were carried in the plasma low density lipoprotein (LDL) fraction than for phylloquinone (9%). The highly lipophilic molecule MK-10 is
almost never detected in plasma though it is frequently the greatest hepatic component. All these results point to the influence of molecular structure and presumably lipophilicity on the tissue distribution and plasma transport of K vitamins.

IV. HEALTH VERSUS DISEASE - METABOLISM AND STATUS

To date the main window of insight into possible changes in the metabolism and status of vitamin K which may reflect or indeed influence various states of health and disease has come from plasma measurements of the vitamin. Compared to other fat soluble vitamins, such measurements are not well established and quite large interlaboratory differences exist [1,4].

Values for plasma K, measured in our laboratory in various studies particularly in conditions with known disturbances of lipid transport and metabolism are shown in Table 1.

TABLE 1

Mean plasma phylloquinone concentrations in health and disease

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Plasma $K_1$(ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Healthy newborn (cords)</td>
<td>20</td>
<td>0.02</td>
</tr>
<tr>
<td>Healthy adults (fasting)</td>
<td>45</td>
<td>0.41</td>
</tr>
<tr>
<td>Healthy adults (non-fasting)</td>
<td>22</td>
<td>0.66</td>
</tr>
<tr>
<td>Hypercholesterolaemia (fasting)</td>
<td>10</td>
<td>0.76</td>
</tr>
<tr>
<td>Hypertriglyceridaemia (fasting)</td>
<td>7</td>
<td>2.55</td>
</tr>
<tr>
<td>Mixed hyperlipidaemia (fasting)</td>
<td>13</td>
<td>4.60</td>
</tr>
<tr>
<td>Diabetes* (normal lipids, fasting)</td>
<td>42</td>
<td>0.62</td>
</tr>
<tr>
<td>Diabetes* (raised lipids, fasting)</td>
<td>38</td>
<td>0.88</td>
</tr>
<tr>
<td>Haemodialysis patients (fasting)</td>
<td>20</td>
<td>0.74</td>
</tr>
</tbody>
</table>

* Untreated non-insulin dependent

The results clearly show the influence of other lipids on the plasma levels of phylloquinone. In healthy adults this results in higher plasma levels in non-fasting compared with fasting subjects. In primary hyperlipidaemia extremely high plasma levels are found in the fasting state particularly in hypertriglyceridaemia whether alone or in combination with hypercholesterolaemia (mixed hyperlipidaemia). Patients with discrete hypercholesterolaemia also have raised $K_1$ levels though this is less marked than in hypertriglyceridaemia. Diseases in which secondary hyperlipidaemia is a common problem also tend to have higher than normal plasma $K_1$ concentrations. This was evident in both diabetic and haemodialysis patients (Table 1).

These results suggest that if plasma $K_1$ measurements are to be used as an index of vitamin K status, and there is some evidence that they can [1], it may be important to take account of plasma lipids, particularly triglycerides. It is suggested that the ratio of plasma $K_1$ to plasma triglycerides would be a better index of status [1,6].
The converse of this association of high plasma K values with raised plasma lipids is seen in the newborn where extremely low levels of K are found in cord blood. Although this is commonly documented as evidence of a poor vitamin K status it should be remembered that plasma lipoproteins at birth are very low so that plasma levels will tend to underestimate the true vitamin K status. For example although we found that plasma levels of K in the newborn were about 30 fold lower than adults the liver stores of the newborn were only about 5 fold lower [1,4].

Although we had previously shown that the great majority of the high plasma levels of K in primary hyperlipidaemia is carried with VLDL very little is known about the transport of vitamin K in healthy subjects or secondary hyperlipidaemia. Our recent studies in haemodialysis patients have revealed some interesting associations. Thus significant positive correlations were found between plasma K and total triglycerides (r=0.73), VLDL triglycerides (r=0.72), VLDL cholesterol (r=0.67) and a negative correlation with HDL cholesterol (r=-0.49). The results confirm the importance of VLDL in the plasma carriage of phylloquinone and preliminary studies suggest that this lipoprotein also plays a key role in the transport of K in healthy subjects. A significant association between plasma K and plasma triglycerides in a large normal population has been reported previously [5]. The lack of a significant correlation with total plasma cholesterol may result from our finding of opposing positive and negative associations of K with cholesterol in VLDL and HDL fractions respectively. The physiological mechanisms behind these findings remain to be clarified.

REFERENCES