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Phylloquinone Content from Wild Green Vegetables May Contribute Substantially to Dietary Intake

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ABSTRACT

Background: Traditional Nordic eatable wild plants are now sold in local stores and available to everyone. Wild vegetables may contain large amounts of vitamin K1. Due to the concomitant therapeutic use of anticoagulants among the populations, it is important to gain knowledge about the content of vitamin K1 in these products, as well as their contribution to the diet. The objective of this study was to measure the vitamin K1 content in four wild eatable plants and to estimate how much these wild vegetables contribute to the daily dietary vitamin K1 intake. Results: The wild vegetables had a high phylloquinone content of 400-600 µg vitamin K1/100 g fresh weight. The average daily intake when consuming the average Danish diet is low (64 ±20 µg/d or 72±23 µg/10 MJ/d), however, inclusion of wild vegetables as in the New Nordic Diet increases the vitamin K1 intake to 23±3±51 µg/d or 260±50 µg/10 MJ/d. Conclusion: Inclusion of more wild vegetables may substantially increase the intake of vitamin K, which could pose a risk for people treated with vitamin K antagonists (VKAs), but may be beneficial for the remaining population.

Keywords: Vitamin K1, Wild plants, Diet, Vitamin K antagonists, Humans, Dandelion, Ramson, Stinging nettle, Ground elder.

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1. INTRODUCTION

Oral vitamin K antagonists (VKAs) are anticoagulants drugs that reduce the body’s ability to form blood clots. They are used for management of several cardiovascular disorders and conditions such as atrial fibrillation, but also for artificial heart valve, deep vein thrombosis, pulmonary embolism, and for prevention of blood clots e.g. genetic clotting disorders in the prevention and treatment of thrombosis. It is estimated that 1.5-2% of the population has atrial fibrillation [1]. Vitamin K antagonists belong to the group of most
frequently used drugs worldwide [2] and are effective anticoagulants because they reduce the regeneration of vitamin K by inhibiting the vitamin K epoxide reductase enzymes in the vitamin K cycle [3]. Patients taking VKAs may be unable to regenerate vitamin K and therefore have impaired clotting factor synthesis, which makes them likely to be critically dependent on a stable oral intake of the vitamin [4]. A reduction in intake of vitamin K can amplify the effect of the drug and cause bleeding, whereas an increased intake of vitamin K can reduce the efficacy of the drug and cause thromboembolism [5]; [6]. In light of this knowledge it is important to make accurate assessments of dietary intake of vitamin K. Vitamin K₁ is the predominant dietary source of vitamin K and can be found in all photosynthetic plants [7]. Many commonly used green vegetables contain large amounts of vitamin K₁.

Cultivated green vegetables like, Broccoli (Brassica oleracea var. Italica), kale (Brassica oleracea var. Sabellica), spinach (Spinacia oleracea) and Brussels sprouts (Brassica oleracea var. Gemnifera) contain approximately 250-560 mg/100 g [8]. Unlike other fat-soluble vitamins vitamin K stores are rapidly depleted if intake is insufficient [9] which may suggests that vitamin K status is influenced by recent intake. Studies show that a consistent intake of vitamin K could reduce intrapatient variability in anticoagulation response and thus improve the safety of VKA therapy [4]. 

Average intake of vitamin K has been estimated in some studies in the past and the mean intake seem to range from approximate 60-250 µg/d [10]; [11] (no recommended intakes currently exist for vitamin K in the Nordic Countries, only a guideline for adults of 1µg/kg body weight per d [12]). An intake of more than 250 µg/d (consumed as 50 g of spinach, 100 g broccoli, 200 g lettuce or cabbage per day), has in one study with patients receiving VKA treatment, been associated with VKA resistance [13].

Contrary to the patients receiving VKA treatment, the present habitual intake of vitamin K have by some been suggested to be too low and should be increased due to the proposed health beneficial effects of vitamin K in relation to bone, cardiovascular diseases and cancer [14]; [15].

These days, much research and publicity is being given to the "New Nordic Diet" and with this an introduction of new wild green leafy vegetables, as ground elder (Aegopodium podagraria), stinging nettles (Urtica dioica), ramsons (Allium ursinum) and dandelion (Taraxacum officinale). Traditional Nordic eatable wild plants are now sold in local super markets and grocery stores and available to everyone. Typically the dark green vegetables as broccoli, Brussels sprout, kale and spinach contain large amounts of vitamin K₁, but also wild, eatable plants my contain a considerably amount and its activity is very important to examine due to the wide spread use as food ingredients and the concomitant therapeutic use of anticoagulants among the populations, as well as the contribution of vitamin K₁ to the general public.

Precise vitamin K₁ content of many dietary components is lacking from food composition databases. The objective of this study was to study the vitamin K₁ content in four wild eatable plants; stinging nettle, dandelion, ramson, and ground elder, each sampled at four different places in Denmark. In addition, data on intake of vitamin K₁ from a previous study on the New Nordic Diet containing these eatable plants was recalculated.

2. MATERIALS AND METHODS

2.1. Plants

The eatable wild plants; stinging nettle, dandelion, ramson, and ground elder, were each sampled at four different places in Denmark. Stinging nettle was collected in Vestskaoven, Hadsund, Amager NV and Amager N. Dandelion was collected in Gentofte, Hadsund, Amager NV and Amager N. Ramson was collected in Bælum, Aarhus, Copenhagen K and Korsør. Ground elder was collected in Gentofte,
Hadsund, Amager Ø and Amager NV. The sampling took place from June to September, and the amount collected for each sample was 70-220 g. The samples were stored at max. -20°C for up to 3 months.

2.2. Analytical Methods

The analytical method for vitamin K applied was previously described in details [16]. In short the collected wild plants was freeze-dried, and 0.2 gram freeze-dried sample was taken for analysis and added vitamin K1-d7 (5,6,7,8-d₄, 2-methyl-d₃) as internal standard. Followed by extraction by n-heptane:ethyl acetate using accelerated solvent extraction, and cleaned-up by silica solid phase extraction. For separation and quantification was used liquid chromatography and mass spectrometry (LC-MS/MS). The precision estimated as internal reproducibility in spinach was 5.2% at the content of 247 µg phylloquinone/100g fresh weight (FW).

2.3. Dietary Intake

Vitamin K₁ content of stinging nettle, dandelion, ramson, and ground elder was added to data on dietary intake of these eatable plant form a previous study of the New Nordic Diet [17]. In the original calculation spinach replaced stinging nettle. Detailed data on dietary intake based on products handed out from a study shop during 26 weeks of intervention made it possible to update the vitamin K₁ content of these eatable plants, and recalculate the vitamin K₁ intake during the intervention.

3. RESULTS

The wild vegetables, stinging nettle, dandelion, ramson, and ground elder, had a high phylloquinone content of 400-600 µg vitamin K1/100 g fresh weight (Table 1). For comparison food database values of vitamin K₁ content in common green cultivated vegetables are given in Table 2.

Recalculation of dietary intake data resulted in a significant higher vitamin K₁ intake of 3 µg/day (p<0.001) among participants eating according to the New Nordic Diet (Table 3).

4. DISCUSSION

The major finding of this study is that the wild vegetables, stinging nettle, dandelion, ramson, and ground elder had a high vitamin K₁ content of 400-600 µg/100 g FW, and therefore can be important sources of intake from the diet. For comparison only spinach (and parsley) has comparably high concentrations of vitamin K₁ [18]. A previous study has found similar concentrations of Vitamin K₁ content in stinging nettles sampled in North Dakota, US. The concentration was 499 µg/100 g FW [19]. Similarly high concentrations of vitamin K₁ has also been found in traditional Asian green vegetables [20]. As can been seen from the table 3, the average daily intake when consuming the average Danish diet is low (64 ±20 µg/d or 72±23 µg/10 MJ/d), however, inclusion of wild vegetables as in the New Nordic Diet increases the average vitamin K₁ intake to 233±51 µg/d or 260±50 µg/10 MJ/d. The majority of the vitamin K₁ intake was due to intake of celery, potatoes, spinach, peas and parsley, both before and after re-calculation of the data. However, the eatable wild plants contributed significantly after re-calculation of the data and support the increased vitamin K₁ intake from wild eatable plants. The low intake of vitamin K₁ in the average Danish diet originated primarily from intake of broccoli, lettuce, potatoes and spinach.

These data suggest that inclusion of more wild vegetables substantially increase the intake of vitamin K, which could pose a risk, if not taken into account, for people treated with VKA. The average intake in the present study show that is it easy to reach the proposed excess of > 150 µg vitamin K₁/daily which may
interfere with anticoagulant therapy in healthy subjects [21] or the excess of > 250 µg/d which has been shown to counteract VKA treatment in patients commencing VKA treatment [13]. However, these estimated maximum doses are based on intake of pure supplemental vitamin K\textsubscript{1}. Vitamin K\textsubscript{1} from green leafy vegetables is considered much less bioavailable, and bioavailability of vitamin K\textsubscript{1} from spinach is only about 5-15% of that from pure vitamin K\textsubscript{1} preparations. Therefore the bioavailability of vitamin K\textsubscript{1} from the wild vegetables needs to be considered when interpreting their likely effect on VKA treatment. While single meals of spinach or broccoli containing 1000-1500 µg vitamin K\textsubscript{1} and 500-700 µg vitamin K\textsubscript{1}, respectively, only affects anticoagulation treatment transiently and non-clinically relevant, continuous daily intakes of vitamin K\textsubscript{1} from foods in these doses require dose adjustment of the anticoagulant therapy [21]; [22].

The average daily intake of vitamin K\textsubscript{1} from the present average Danish Diet seems to be around 60-70 µg/d, no previous Danish data on dietary vitamin K\textsubscript{1} exists. Although no ADI is available, the Nordic Nutrition recommendations recommends a daily intake of 1µg/Kg/day, so the estimated average intake is probably somewhere in the lower end. Hepatic vitamin K insufficiency in the Danish population is not a problem; however, concern could be raised regarding risks arising from long term insufficient intakes of vitamin K e.g osteoporosis, cardiovascular diseases and cancer due to the extra-hepatic functions of vitamin K. It has been suggested that for an optimal function of vitamin K in extra-hepatic tissues, much higher amounts are needed than for optimal hemostasis [15]. So for the healthy Danish population inclusion of more wild vegetables would probably be beneficial, while patients treated with VKA should be more careful about changing dietary habits without consulting their doctor.

The current obtained values can be incorporated into food tables and databases, and should also be available for caregivers and patients receiving VKA.

**Table-1.** Phylloquinone (vitamin K\textsubscript{1}) content in four wild vegetables. For each species the sampling (N=4) was performed at four different locations in Denmark and the phylloquinon content assessed by LC-MS/MS.

<table>
<thead>
<tr>
<th></th>
<th>µg vitamin K\textsubscript{1}/ 100g fresh weight</th>
<th>Mean</th>
<th>RSD, %</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stinging nettle</td>
<td>600</td>
<td>22</td>
<td>434-751</td>
<td></td>
</tr>
<tr>
<td>Dandelion</td>
<td>466</td>
<td>21</td>
<td>386-591</td>
<td></td>
</tr>
<tr>
<td>Ramson</td>
<td>397</td>
<td>7</td>
<td>374-424</td>
<td></td>
</tr>
<tr>
<td>Ground elder</td>
<td>532</td>
<td>13</td>
<td>440-605</td>
<td></td>
</tr>
</tbody>
</table>

**Table-2.** The vitamin K\textsubscript{1} content in cultivated green vegetables [8].

<table>
<thead>
<tr>
<th></th>
<th>µg vitamin K\textsubscript{1}/ 100g fresh weight</th>
<th>Mean</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Broccoli</td>
<td>260</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brussel sprouts</td>
<td>250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kale</td>
<td>250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinach</td>
<td>560 (replaced stinging nettle in the original calculations)</td>
<td>560</td>
<td></td>
</tr>
</tbody>
</table>

Source: From the Danish Food composition database [8].
Table 3. Vitamin K\textsubscript{1} content in an average "average Danish Diet" (ADD) and in an average "New Nordic Diet" (NND). Mean (SD) Energy, mass and vitamin K intake. Data are based on total food handed out from an experimental research shop to 147 volunteers during 26 weeks of dietary intervention\cite{17}.

<table>
<thead>
<tr>
<th></th>
<th>NND (n=91)</th>
<th>ADD (n=56)</th>
<th>NND vs ADD p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ/day)</td>
<td>9.049 (1617)</td>
<td>9.025 (1658)</td>
<td>0.93</td>
</tr>
<tr>
<td>Vitamin K\textsubscript{1} (μg/10MJ)</td>
<td>257 (49)</td>
<td>72 (23)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vitamin K\textsubscript{1} recalculated (μg/10MJ)</td>
<td>260 (50)**</td>
<td>72 (23)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vitamin K\textsubscript{1} (μg/day)</td>
<td>230 (51)</td>
<td>64 (20)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vitamin K\textsubscript{1} recalculated (μg/day)</td>
<td>233 (51)**</td>
<td>64 (20)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\* p-value by students t-test

** Recalculated data of vitamin K\textsubscript{1} significantly different from original data (paired t-test)

Authors Contributions

AA, CS and JJ designed the present study. JJ conducted the food analysis. SKP prepared the dataset. SB, CS, JJ and SKP analyzed data and wrote the paper. SB had primary responsibility for the final content. All authors read and approved the final manuscript.

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