Cereal grains, alpha tocotrienol and cholesterol metabolism in the rat

G.H. McIntosh BVSC, PHD, F.H. Bulman ASSO DIP APPL SC and G.R. Russell BSC†

CSIRO Division of Human Nutrition, Kintore Avenue, Adelaide, South Australia 5000, Australia.

The influence of alpha (α)-tocotrienol, the main vitamer of vitamin E in barley and oats, on cholesterol synthesis has been studied in laboratory rats. Both oats and barley lowered plasma cholesterol relative to wheat, which had no such effect, and the change has been attributed to an inhibitory influence of α-tocotrienol on cholesterol synthesis rate.

Vitamin E was stripped from oats and barley by a petroleum ether extraction procedure and the grains compared with their unstripped equivalents. In the oats feeding experiment this resulted in a higher plasma cholesterol and lower liver cholesterol synthesis rate. The barley experiment produced no significant response.

Pure α-tocotrienol was gavaged into rats fed a semipurified diet without vitamin E, at the rate of 380 µg/rat/day for 28 days. There was no significant influence on plasma cholesterol level or on liver cholesterol synthesis rate. From these studies it is concluded that α-tocotrienol does not influence cholesterol synthesis rate significantly. Therefore, it is unlikely to be a factor in oats and barley responsible for the plasma cholesterol lowering observed.

Introduction

The potential of cereal grains such as barley and oats to lower plasma cholesterol concentration relative to wheat has been attributed to several factors, one such factor being their β-glucan (dietary fibre) content1-4. A lipid-solvent-soluble component in high-protein barley flour was also active in this respect and the identification of one factor as α-tocotrienol has raised considerable interest in the potential of this biologically active isomer to influence cholesterol metabolism5. Similar reports using rodents and chickens have appeared previously establishing an ability of α-tocopherol to lower plasma lipids6-7-8.

These studies then were aimed at examining the influence of α-tocotrienol on cholesterol metabolism in the laboratory rat against the background of a predominantly grain diet. Because the stripping of vitamin E isomers may include unidentified components, or lead to other changes in the grain, a pure α-tocotrienol dosing experiment was also undertaken in order to detect any likely cholesterologenic effect of α-tocotrienol, not explainable by other components of the grain. These studies dispel the likelihood of α-tocotrienol having a significant cholesterol synthesis inhibitory role, at least in the laboratory rat. They suggest that other lipid solvent soluble factors may be involved to explain the oat diet observation. Secondly, the lowering of plasma cholesterol by oats and barley is probably more a result of the dietary fibre component β-glucan, which both grains have in common.

Materials and methods

The experiments were devised to assess the dietary influence of cereal grains, barley, wheat and oats, and in particular their content of vitamin E on cholesterol metabolism. Diets were prepared for feeding experimental rats based on 75% whole grain with the addition of 15% skimmed milk powder, 1% sugar, 2% sunflowerseed oil, 2% lard and 5% trace elements, minerals and vitamins as for the AIN76 diet9. In experiment 1, barley and oats were used in a direct comparison of their influence on cholesterol metabolism. Both barley and oats had the hulls removed (lightly pearled), thereby enabling a direct comparison with wheat. In experiments 2 and 3, the aim of the studies were to compare the vitamin E stripped crushed grains, barley and oats, with their unstripped equivalent. This provided a means of assessing α-tocopherol and also α-tocotrienol influence on cholesterol metabolism. Barley, wheat and oats were used and vitamin E was extracted from the barley and oats by soaking the crushed grain in petroleum ether (40-70°C BP), rotary evaporating to retrieve the lipids and removing vitamin E was used with success in a previous study10. In the case of oats the oil extracted was a significant fraction (6% by weight) and was therefore added back after removal of the charcoal. In the case of barley, stripped corn oil because it had a fatty acid profile very similar to that of barley oil, was added back at 2% to replace the fat extracted.

The vitamin E concentration measured by HPLC12 in oats was 5.6 ppm α-tocopherol and 20.4 ppm α-tocotrienol, and for barley was 11.5 ppm α-tocopherol and 27 ppm α-tocotrienol. Wheat was 5.5 ppm α-
tocopherol and 1.3 ppm α-tocotrienol. Vitamin E stripping of oats reduced the concentration of α-tocopherol from 5.6 to 1.2 ppm and α-tocotrienol from 20.4 to 4.4 ppm. These are equivalent to deficiency diet levels. Vitamin E stripping of barley reduced the α-tocotrienol level from 27 to 2.8 ppm and α-tocopherol level from 11.5 to 0.6. The final diet as fed contained 0.2 ppm α-tocotrienol and 2.8 ppm α-tocopherol.

In experiment 4, the influence of α-tocotrienol was assessed directly using a pure source of α-tocotrienol (αT3) (supplied by the Eisai Co., Tokyo) gavaged into rats fed a semipurified diet, but excluding the addition of vitamin E (α-tocopherol). For this experiment they were fed a rat diet consisting of 64% wheat flour 15% skimmed milk powder 8% sucrose 8% cellulose 2% sunflower seed oil and 3% trace elements minerals and vitamins (AIN76). This diet on assay was found to contain 3 ppm vitamin E as α-tocopherol.

The experimental diets were fed to inbred hooded Wistar rats aged 4–8 weeks and weighing between 155 and 240 g. The diets were fed for periods of 4 weeks after which the rats were killed. The rats ingested on average 29 ± 2 g feed per day. In the gavaging experiment (expt 4), the α-tocotrienol was given orally to each rat at the rate of 380 μg daily for a period of 28 days. Control rats were gavaged with the vehicle which was glycerol (0.5 g daily).

To assess cholesterol synthesis rate in the intestine and liver of rats, the titrated water method of Jeske and Dietzsch13,14 was used. This involved the injection of 5 mCi 3H2O in 0.5 ml saline intraperitoneally 1 h before rats were anaesthetized with diethylether, and blood removed into an EDTA container. Plasma was used for triglyceride and total cholesterol concentrations, vitamin E assay, and tritium counting for specific activity of water in plasma. The liver was perfused with 50 ml cold saline to wash out any residual blood, and the liver removed blotted dry and weighed. It was then chopped up finely and dropped into 40 ml ice-cold methanol and homogenized. The small intestine was dissected out, washed empty of contents and treated similarly. Total fat extracts were made from these homogenates by adding chloroform and centrifuging to separate off protein. This fat extract was hydrolysed for (1) vitamin E, (2) total cholesterol and (3) tritium-labelled cholesterol synthesis determination.

(1) For vitamin E assay the fat extract was dissolved in 5 ml ethanol containing 3% pyrogallol and 0.05% BHT. One ml of 10% KOH was added under nitrogen, the tube resealed and heated at 60°C for 30 min, cooled to 0°C and 6 ml water added. This was then extracted with 40–60°C pet ether, the extract evaporated to dryness with nitrogen and the residue redissolved in 5 ml methanol for HPLC assay using fluorimetric detection.

(2) A similar procedure was used for the assay of total cholesterol except that the residue after evaporating with nitrogen was dissolved in chloroform for GLC assay15.

(3) For cholesterol synthesis determination the fat extract was hydrolysed as before and the cooled extract shaken with pet ether. The pet ether was evaporated to dryness and the residue dissolved in chloroform. This fraction was run on TLC plates of silica 60H in a 1-cm side band. The solvent was pet ether 180 : ether 30 : acetic acid 2. The cholesterol spot was cut out and added to ASC II (Amersham) counting medium for 3H counting. A diluted aliquot of plasma was also counted in ASC II. Plasma is 91% water, so specific activity of water could be calculated. Cholesterol synthesis rate, expressed as incorporation into cholesterol of μmol 3H2O per g liver or intestine per hour. This was calculated from specific activity of body water and the radioactivity of isolated cholesterol.

Results were analysed statistically using Students’ t-test.

Results

The results for four experiments are summarized in Tables 1 and 2. Table 1 showing body and liver weights and liver cholesterol synthesis. There were no significant body or liver weight differences between treatments in any of the experiments. There were however much larger gains in body weights in experiments 2 and 3 with barley than with oats. The influence of treatments on cholesterol synthesis are also shown. Only in experiment 2 was there a marked change in liver cholesterol synthesis, the vitamin E stripped oat diet having a lower cholesterol synthesis rate than the unstripped sample (down 27%, P < 0.05). In none of the other treatments was cholesterol synthesis in the liver affected. However, cholesterol synthesis in the small intestine was increased in experiment 4 (6.23 ± 0.18 to 7.17 ± 0.26 μmol 3H2O/g/h, ie 15% P < 0.05) with the gavaging of α-tocotrienol into the rats. This was associated with an actual reduction in cholesterol content (1.75 to 1.40 mg/g).

There was a significant reduction in plasma cholesterol (14%, P < 0.05) in rats fed the oat and barley diets relative to the wheat-based control diet (Table 2, experiment 1). By comparison the plasma triglyceride concentration increased with oats (+10%) and barley (+26%) feeding, although there was considerable variability in the data and these differences were not significant statistically. Liver cholesterol concentration showed a small increase with the oat-based diet and fell with the barley-based diet relative to the wheat diet. Neither cat nor barley groups however showed any significant difference from the wheat group (experiment 1).

The influence of stripping vitamin E is shown in the second and third experiments (Table 2). With vitamin E removed from oats there was an increase in plasma cholesterol (+11%) and a decrease in liver cholesterol concentration (~15%, P < 0.01), associated with a decrease in liver cholesterol synthesis rate.

Plasma triglyceride concentration fell (44% ns). However, with barley, no such changes were produced with vitamin E stripping, this despite an effective lowering of plasma and liver α-tocotrienol concentrations (Table 2).

The influence of supplementing rats on a semi-purified diet (AIN76 but without vitamin E) with α-tocotrienol is shown in experiment 4. There was no significant change in liver cholesterol synthesis, nor in the liver or plasma lipids associated with a significant
rise (five-fold) in α-tocotrienol concentration. Alpha-
tocopherol in plasma was low and showed no change,
indicating evidence of a small deficit. The influence of
vitamin E status of the diets on plasma and liver levels
of α-tocopherol and α-tocotrienol is also shown in Table
2. It is apparent that alterations in α-tocotrienol and α-
tocopherol in the grains were significantly reflected in
the plasma and liver concentrations of these vitamers.
In experiment 1, the plasma α-tocopherol concentration
changed in the opposite direction to α-tocotrienol,
increasing with the decreasing concentration of α-
tocotrienol from oat- to barley- to wheat-based diets,
the latter containing virtually no α-tocotrienol.

The stripping of vitamin E was effective at lowering
plasma and liver levels, but was less effective for the oat-
than for the barley-based diet. The situation may
have been complicated by the higher level of fat in oats
than barley.

The gavaging of α-tocotrienol in experiment 4 was
without effect on the liver α-tocotrienol value, but sig-
ificantly increased plasma α-tocotrienol and α-
tocopherol in the liver.

Discussion
Considerable interest surrounds the potential for bio-
logical control of cholesterol synthesis\(^6\). The purported
ability of α-tocotrienol to significantly alter cholesterol
synthesis rate has been tested in these studies in the
laboratory rat, the vitamin E forms used being presented
as the pure isolated form and as the major vitamin E
component of oats and barley, but not of wheat. In neither
approach was there any significant decrease in choles-
terol synthesis rate in the liver associated with an in-
creased α-tocotrienol concentration. Indeed the opposite
response was apparent. Where vitamin E levels were
lowered by removal from the diet, the cholesterol syn-
thesis rate and liver cholesterol levels were lower than
the matched controls. In the oats experiment (expt 2
the liver cholesterol synthesis data showed a greater
rate when α-tocopherol and α-tocotrienol were present.
However, this did not occur in the barley study (expt 3),
nor was it seen with α-tocotrienol gavaging for 28 days.
One possible interpretation of this experiment is that
inadequate levels of α-tocotrienol were gavaged to pro-

Table 1. Commencing and final body and liver weights and liver cholesterol synthesis rates in experimental rats after 4 weeks
on experimental diets.

<table>
<thead>
<tr>
<th>Expt 1</th>
<th>Commencing weight (g)</th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
<th>Liver cholesterol synthesis (μmo lipid/g/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearled oats</td>
<td>229.8±3.9 (8)</td>
<td>11.5±1.7</td>
<td>0.71±0.18</td>
<td></td>
</tr>
<tr>
<td>Pearled barley</td>
<td>155±2.2² (24)</td>
<td>233.0±4.9 (8)</td>
<td>12.7±1.0</td>
<td>0.70±0.12</td>
</tr>
<tr>
<td>Wheat</td>
<td>223.0±3.1 (8)</td>
<td>12.2±1.1</td>
<td>0.68±0.24</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Expt 2</th>
<th>Oats (-Vit E)</th>
<th>240.0±2.3 (16)</th>
<th>260.0±2.5 (8)</th>
<th>10.0±0.7</th>
<th>1.29±0.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oats (+Vit E)</td>
<td>261.5±3.8 (8)</td>
<td>9.3±0.3</td>
<td>1.77±0.32</td>
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<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Expt 3</th>
<th>Barley (-Vit E)</th>
<th>240.0±3.8 (16)</th>
<th>345.5±5.2 (8)</th>
<th>16.3±0.7</th>
<th>0.85±0.11</th>
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<tbody>
<tr>
<td>Barley (+Vit E)</td>
<td>348.0±5.0 (8)</td>
<td>17.9±0.5</td>
<td>0.82±0.11</td>
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</table>

<table>
<thead>
<tr>
<th>Expt 4</th>
<th>Gavage (-αT3)¹</th>
<th>204.0±3.0 (16)</th>
<th>227.7±2.5 (8)</th>
<th>8.8±0.4</th>
<th>0.56±0.08</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gavage (+αT3)</td>
<td>230.2±4.1 (8)</td>
<td>8.8±0.5</td>
<td>0.60±0.06</td>
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<td></td>
</tr>
</tbody>
</table>

( ) = number of rats per group.
* Students t-test P<0.05.
¹αT3 (alpha-tocotrienol) was gavaged at 380 µg/rat/day.
²Mean ± SEM.
αT3 α-tocotrienol.
Vit E α-tocotrienol and α-tocopherol.
Table 2. Cholesterol and triglyceride and vitamin E (α-tocopherol and α-tocotrienol) values in liver and plasma of experimental rats.

<table>
<thead>
<tr>
<th></th>
<th>Vitamin E</th>
<th>Lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Plasma</td>
</tr>
<tr>
<td></td>
<td>αT (3)</td>
<td>αT</td>
</tr>
<tr>
<td></td>
<td>(μg/g)</td>
<td>(μg/100ml)</td>
</tr>
<tr>
<td><strong>Expt 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pearled oats</td>
<td>0.94±0.28</td>
<td>66.0±6.2</td>
</tr>
<tr>
<td>Pearled barley</td>
<td>0.4±0.11</td>
<td>37.0±3.0</td>
</tr>
<tr>
<td>Wheat</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Expt 2</strong></td>
<td></td>
<td></td>
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<tr>
<td>Oats (−Vit E)</td>
<td>0.08±0.0</td>
<td>41.3±3.3</td>
</tr>
<tr>
<td>Oats (+Vit E)</td>
<td>1.04±0.19</td>
<td>101±10.6</td>
</tr>
<tr>
<td><strong>Expt 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barley (−Vit E)</td>
<td>0</td>
<td>17.0±2.5</td>
</tr>
<tr>
<td>Barley (+Vit E)</td>
<td>0.99±0.12</td>
<td>96.0±12.5</td>
</tr>
<tr>
<td><strong>Expt 4</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gavage (−αT3)</td>
<td>0.56±0.15</td>
<td>324±12.4</td>
</tr>
<tr>
<td>Gavage (+αT3)</td>
<td>0.46±0.13</td>
<td>356±15.6</td>
</tr>
</tbody>
</table>

*: Indicates statistical significance at \(P<0.05\) or less. Each value represents the mean and standard error for eight rats. αT3 alpha-tocotrienol, αT = alpha-tocopherol.

duce a significant biological dose. However, levels were chosen to be consistent with likely food intake and to accommodate the available supply. When combined with the initial experiment, there is no support for a claim that cholesterol synthesis in experimental rats is significantly reduced by α-tocotrienol from barley or oats. In this respect, these studies do not support the observations of Qureshi and others\(^{17,24}\) with regard to α-tocotrienol on liver cholesterol synthesis in chickens, pigs or isolated rat liver. Hirahara\(^{18}\) examined the influence of larger doses of tocotrienol in rats made hypercholesterolaemic by dietary means, and there was again no significant cholesterol lowering with α-tocotrienol. On the other hand, it has been claimed that large doses of α-tocopherol acetate given orally to rats have proved effective in lowering plasma and liver cholesterol and other lipids\(^{6,7}\). Here again, there is no consistent support for such an observation in these studies. However, in none of these studies have large (pharmacological) changes of vitamin E intake been involved, as are used in some experimental protocols.

Fat or fat solvent soluble factors responsible for a cholesterol lowering effect were also reported\(^{5,19,20}\). In this regard such a factor may have been operating in oats as was observed in these studies. Other possibilities for achieving this effect include the β-glucan component of dietary fibre and a triglyceride containing linoleic and γ-linolenic acids in oats and barley. The latter has been shown to be hypocholesterolaemic\(^{20}\). The adding back of stripped oat oil in that experiment to the diet should have precluded the possibility of linoleic acid rich triglyceride being a significant factor in some way. However, there is a chance that it was in some way damaged by the stripping process, and therefore failed to have any influence.

The tocotrienol and tocopherol levels in plasma and liver reflected the dietary values quite closely in the first three experiments. It was of interest that the α-tocotrienol appeared to be depleted to a greater degree than α-tocopherol, or alternatively the α-tocotrienol was less effectively absorbed. There is no information available on the relative rates of utilization of these vitamers, although their comparative biological activity in bioassay is well established. Alpha-tocotrienol has approximately one-third the activity of α-tocopherol. There was no clinical evidence in these studies of a vitamin E deficiency as a result of the treatments, the plasma levels of α-tocopherol being just above that associated with deficiency in all experiments.

The inverse relationship between α-tocopherol and α-tocotrienol levels in plasma and livers of the oats/barley and wheat fed rats is of interest. This could indicate an antioxidant protective effect between these vitamers, it having been claimed\(^{21}\) that α-tocotrienol is a powerful antioxidant relative to α-tocopherol. Alternatively it may merely represent the dietary concentrations presented by the three grains.

While there is undoubtedly more to be learned regarding the eight vitamers of vitamin E and their influence on metabolism, it would appear from these studies that the influence of α-tocopherol and α-tocotrienol on triglyceride and cholesterol concentration in the rat is insignificant. Whether other species in which significant biological responses have been shown, such as the rabbit and chicken\(^{22,22-25}\), are better models for representing the human situation, only more research will establish. The recent publications by Qureshi and workers\(^{26,27}\) of the influence of α-tocotrienol from palm oil on serum cholesterol in humans and of brewers barley grain on chicken lipid metabolism raise similar questions, with
little resolutions as to which, if any, of the tocotrienols are implicated. The availability of pure forms of tocotrienol (presently unavailable!) could improve the possibility of resolving some of these issues.

Acknowledgements —We wish to acknowledge the technical assistance of Mr Li Gang of Beijing PRC with this work. The α-tocotrienol and other pure standards of vitamin E for assay were a gift from the Eisai Co, Tokyo, Japan. This study was supported by a grant from the Barley Research Council of Australia.

References
作者用大白鼠為試驗對象，研究了維生素E的主要異构体，α-生育三烯酚對膽固醇合成速度的影響。他們認為α-生育三烯酚對膽固醇的合成有抑制作用，燕麥與大麥含有較多的α-生育三烯酚，因而有降低膽固醇的效果，而小麥則沒有這種作用。

他們用石油醚除去燕麥及大麥粒中的維生素E，並與沒有除去維生素E的燕麥及大麥相比較。結果發現喂養去維生素E燕麥的大鼠，其血漿膽固醇升高，而肝臟膽固醇合成下降。喂養大麥的大鼠兩組無明顯差異。

作者再用半純淨不含維生素E的飼料喂養大鼠28天，每天每鼠管飼純α-生育三烯酚380微克，結果發現對血漿膽固醇水平和肝臟膽固醇合成速度無明顯影響。因而得出結論，α-生育三烯酚對膽固醇的合成速度無明顯影響。