Long-term effects of a change from a mixed diet to a lacto-vegetarian diet on human urinary and faecal mutagenic activity

Gunnar Johansson1,4, Anders Holmén2, Lennart Persson3, Benkt Högestedt2, Cecilia Wassén1, Ludmila Ottova1 and Jan-Ake Gustafsson1

1Department of Medical Nutrition, Karolinska Institute, F 60, Novum, Huddinge Hospital, S-141 86 Huddinge, 2Department of Occupational Medicine, Central Hospital, Halmstad and 3Swedish Laboratory Services Ltd, Halmstad, Sweden

This is an investigation of the long-term effects of a shift from a mixed diet to a lacto-vegetarian diet and of a return to a mixed diet on the mutagenic activity in urine and faeces. The participants were 20 normal weight, non-smoking subjects. Dietary surveys and urinary and faecal samples were collected before and 3, 6 and 12 months after the dietary shift as well as 3 years after termination of the lacto-vegetarian diet period. The faecal samples were assayed for direct acting mutagens with the fluctuation test for weak mutagens and the urinary samples were assayed with the same assay but with a metabolic activation system, the so-called S9 fraction. The dietary data showed an increase in consumption of fruits, vegetables and dairy products and a decrease in meat, fish, eggs, sweets and biscuits during the vegetarian diet period. These changes led to an increase in total carbohydrates, fibre, vitamin C and calcium and a decrease in fat and protein intake. Mutagenic activity in both urine and faeces decreased after shift to the vegetarian diet and mutagenic activity in faeces increased when the volunteers returned to a mixed diet (P = 0.025 and 0.035 respectively when comparing the diets). These data indicate that dietary factors may affect mutagenic activity in urine and faeces. However, it is still not clear whether a decrease in animal products, a change in other nutritional factors or a decrease in frying are the main contributors to this change.

Introduction

Dietary factors are known to play an important role in cancer development. For instance, high intakes of total energy and fat enhance cancer development, as well as minor components such as mycotoxin contaminants, nitrosamines, polycyclic aromatic hydrocarbons, plant alkaloids and heterocyclic amines in cooked foods (Ames, 1983; Wakabayashi et al., 1992). Foods also contain substances that protect against cancer development, for instance several vitamins and also crude factors such as total carbohydrates (Wakabayashi et al., 1992; Cassidy et al., 1994). Vegetarian diets have been suggested to be protective against several cancer forms, mainly smoking- and alcohol-related types, but also colon cancer (Phillips, 1975; Phillips and Snowdon, 1985). The latter has been found to be diet related in many studies (Armstrong and Doll, 1975; Phillips, 1975; Correa and Haenzel, 1978; Phillips and Snowdon, 1985; Krepinsky et al., 1986). Faecal mutagenic activity has also been shown to be affected by dietary factors and, in general, so-called high risk groups for colon cancer have higher levels of faecal mutagens than low risk groups (Ehrich et al., 1979; Kuhnlein, U. et al., 1981; Mower et al., 1982).

Cancer is assumed to require a long time (years) to develop and it is therefore important to study long-term effects of various factors, since minor changes in exposure to cancer promoting agents over a long period may have major effects. With this in mind we have performed a long-term dietary shift (1 year) from a mixed diet to a lacto-vegetarian diet to study excretion of mutagens in urine and faeces. We also investigated the volunteers 3 years after termination of the study, when 17 of 20 volunteers returned to a mixed diet.

Materials and methods

Subjects

Twenty volunteers participated in the study (four men and 16 women, 27-61 years of age, mean age 44 years). A call for participants was made by the local press and radio and the volunteers were selected after taking part in a clinical examination together with an interview. They were all healthy, non-smoking omnivores who were not taking any medical drugs and had not taken antibiotics for at least 6 months prior to the start of the study. This study was approved by the Ethical Committee of the University of Gothenburg, Gothenburg, Sweden.

Experimental design

The volunteers were divided into three groups, each of which started their dietary shift at different periods of the year: March, May and August. The subjects participated in a vegetarian cooking course and received lectures in basic nutrition. These courses were held once a week for a period of 3 months. The starting point of the dietary change was defined as the time when the participants commenced these courses and thus started to change their diet. A dietician educated the volunteers with regard to the dietary regimen. The lacto-vegetarian diet served at Swedish Health Centres was used as a guide (Laurell and Loof-Johanson, 1981). This diet is characterized by large amounts of raw vegetables, fruits, unrefined foods and wholemeal products, with the exclusion of meat, poultry, fish and eggs. The consumption of soft drinks, coffee, tea, alcoholic beverages, sweets, sugar, salt and all refined and instant foods was discouraged. Dairy products were allowed, but fermented rather than unfermented products were recommended.

Dietary surveys

The dietary surveys consisted of a repeated 24 h recall method (Callmer et al., 1986), which was carried out before (0 months) and 3, 6 and 12 months after the dietary shift and 3 years after termination of the lacto-vegetarian diet period (48 months). On each occasion four 24 h recalls were completed (covering food intake for a Sunday, Monday, Tuesday and Wednesday respectively), the first being performed as a personal interview in the participant's home, the other three by telephone. Thus each participant was interviewed 5 X 4 = 20 times in all. At the first interview the participant was trained in estimating volumes and weights of foods. The latter three interviews at each time period were carried out without the volunteers' prior knowledge and at irregular intervals. The information given in the interviews was coded and entered into a computer. Energy and nutrient content was calculated using a computer program from the Swedish National Food Administration (Uppsala, Sweden). In the food frequency questionnaire information was collected about meal pattern, food frequencies, cooking methods and food supplements. The food frequencies and the use of different cooking methods were analysed by...
using food frequency points, where one point represents using a cooking method or eating a raw vegetable meal once a month (Johansson et al., 1992a).

**Urinary and faecal sample preparation**

A 24 h urinary sample was collected before (0 months) and 3, 6 and 12 months after the dietary shift and also 3 years after termination of the study (48 months). The urine was filtered through a Whatman no. 1 filter and an aliquot was analysed for creatinine content. The urine (100 ml) was concentrated by passage through a column filled with XAD-2 resin. The adsorbed substances were eluted with acetone and after evaporation the residue was dissolved in DMSO and kept at -70°C until analysis (Falck et al., 1980). The 0, 3, 6 and 12 month samples were analysed shortly after the 12 month samples were collected and the 48 month samples were analysed immediately after termination of the study.

Faecal samples collected from each participant over a 48 h period were obtained at the same time intervals as the urinary samples. The participants stored the faecal samples in their homes in plastic jars in a thermos containing dry ice (-79°C). The thermos flasks were collected and the faecal samples were stored in a freezer (-20°C) until completion of the study. The faecal samples were analysed at the same time intervals as the urinary samples.

**Analysis of mutagens in urine**

Mutagenic activity in urine was analysed according to Falck et al. (1980) with minor modifications. Microtitre plates containing 96 wells were used instead of test tubes (Hubbard et al., 1984). The bacterial strains Escherichia coli WP uvrA and Salmonella typhimurium TA 98 were used in the mutagenic assay, together with an S9 mix. A post-mitochondrial fraction prepared from Aroclor-induced male Sprague-Dawley rats was used as a metabolizing system, the so-called standard liver S9 mix (Maron and Ames, 1983). Three fractions corresponding to 6.2, 3.1 and 1.6 ml urine were tested for mutagenic activity. All samples were analysed in duplicate. No washing of the urinary samples was performed previously shown that the urinary samples contained no tryptophan. The sum of positive wells for the four dilution steps for each time period was used in the statistical analysis (Kuhnlein, H.V. et al., 1983). By positive wells we mean the number of positive wells in the sample minus the number of positive wells in the blank.

**Analysis of mutagens in faeces**

Three grams of faeces (wet weight) were homogenized in 6 ml distilled, deionized water and centrifuged at 4°C for 50 min at 10 000 g. The supernatants were collected and sterilized by successive passage through filters with 0.45 and 0.2 mm pore sizes respectively (Schleicher and Schuell, Dassel, Germany). The extracts were stored at -20°C until analysis. Mutagenic activity in faecal extracts was analysed according to Falck et al. (1980) with minor modifications. Microtitre plates containing 96 wells were used instead of test tubes (Hubbard et al., 1984). The bacterial strains E.coli WP uvrA and S.typhimurium TA 98 were used. The former bacterial strain is prone to base pair mutation and the latter strain is prone to frame-shift mutation. Four fractions corresponding to 3.1, 1.6, 0.8 and 0.4 ml faecal extract were tested for mutagenic activity. All samples were analysed in duplicate. It has been shown that faecal samples contain enough tryptophan to influence the results (Venitt and Bosworth, 1986). We therefore modified our method for 3 h, washed twice and then incubated for 3 days at 37°C (O'Connor et al., 1984). The mutagenic activity was assayed and statistical analysis was performed in the same way as for the urinary samples.

**Statistical analysis**

All tables show the results before the dietary change (0 months) as mean values with a 95% confidence interval (± 2 SEM). The values at 3, 6, 12 and 48 months in Tables II and III are given relative to the 0 month value, which was set to 100%.

**Results**

The major trends when changing from the mixed to the lacto-vegetarian diet were an increase in consumption of fruits, berries, vegetables, herbal tea and dairy products and a decrease in intake of biscuits and buns, sweets, alcoholic beverages, coffee and tea (Table I). The intake of fish, eggs and meat products decreased to zero at 3 months and remained at zero at 6 months. At 12 months two subjects ate small amounts of these animal products. At 48 months 17 of 20 subjects had returned to a mixed diet.

**Table I. Consumption of various food items before (0 months) and 3, 6 and 12 months after dietary shift and 3 years (48 months) after the end of the diet period**

<table>
<thead>
<tr>
<th>Food group</th>
<th>Time (months after dietary shift)</th>
<th>Mixed diet</th>
<th>Lacto-vegetarian diet</th>
<th>Mixed diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>3</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Meat, fish, eggs</td>
<td>161 ± 34</td>
<td>0</td>
<td>1</td>
<td>63</td>
</tr>
<tr>
<td>Vegetables</td>
<td>161 ± 40</td>
<td>224</td>
<td>220</td>
<td>248</td>
</tr>
<tr>
<td>Fruits, berries</td>
<td>279 ± 70</td>
<td>167</td>
<td>148</td>
<td>188</td>
</tr>
<tr>
<td>Root vegetables</td>
<td>147 ± 41</td>
<td>150</td>
<td>156</td>
<td>168</td>
</tr>
<tr>
<td>Milk, 0.5–3% fat</td>
<td>461 ± 95</td>
<td>120</td>
<td>101</td>
<td>104</td>
</tr>
<tr>
<td>Cheese</td>
<td>39 ± 18</td>
<td>185</td>
<td>187</td>
<td>182</td>
</tr>
<tr>
<td>Cream, 12–40% fat</td>
<td>5 ± 4</td>
<td>420</td>
<td>560</td>
<td>480</td>
</tr>
<tr>
<td>Cereals</td>
<td>243 ± 38</td>
<td>148</td>
<td>137</td>
<td>132</td>
</tr>
<tr>
<td>Biscuits</td>
<td>98 ± 30</td>
<td>18</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>Coffee</td>
<td>579 ± 132</td>
<td>3</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Tea</td>
<td>152 ± 108</td>
<td>6</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Herbal tea</td>
<td>12 ± 14</td>
<td>291</td>
<td>358</td>
<td>577</td>
</tr>
<tr>
<td>Alcoholic beverages</td>
<td>62 ± 41</td>
<td>21</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Sweets</td>
<td>5 ± 5</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>

The results before the dietary shift are presented as mean values with a 95% confidence interval (± 2 SEM) of the food items/10 MJ and the values at 3, 6, 12 and 48 months are presented as percentages of the 0 month value (n = 20).

**Table II. Energy and nutrient intake before (0 months) and 3, 6 and 12 months after dietary shift and 3 years (48 months) after the end of the diet period**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time (months after dietary shift)</th>
<th>Mixed diet</th>
<th>Lacto-vegetarian diet</th>
<th>Mixed diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>3</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>7960 ± 950</td>
<td>95</td>
<td>87</td>
<td>82</td>
</tr>
<tr>
<td>Protein (g/10 MJ)</td>
<td>86 ± 6</td>
<td>91</td>
<td>88</td>
<td>87</td>
</tr>
<tr>
<td>Fat (g/10 MJ)</td>
<td>95 ± 5</td>
<td>89</td>
<td>88</td>
<td>85</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>293 ± 11</td>
<td>112</td>
<td>113</td>
<td>116</td>
</tr>
<tr>
<td>Fibre (g/10 MJ)</td>
<td>25 ± 3</td>
<td>168</td>
<td>172</td>
<td>176</td>
</tr>
<tr>
<td>Vitamin C (mg/10 MJ)</td>
<td>115 ± 28</td>
<td>165</td>
<td>125</td>
<td>187</td>
</tr>
<tr>
<td>Calcium (mg/10 MJ)</td>
<td>1250 ± 120</td>
<td>135</td>
<td>125</td>
<td>126</td>
</tr>
</tbody>
</table>

The results before the dietary shift are presented as mean values with a 95% confidence interval (± 2 SEM) of the food components/10 MJ and the results at 3, 6, 12 and 48 months are presented as percentages of the 0 month value (n = 20).

Energy intake decreased throughout the vegetarian period and increased again 3 years after termination of the study, compared with the 12 month values (Table II). Nutrient intake showed the greatest change between 0 and 3 months. Between 3, 6 and 12 months after the dietary shift there were only minor changes. At 48 months the values had returned towards the 0 month values. [See Johansson et al. (1992a,b) for a more detailed dietary description.]

Faecal wet weight increased 3 months after the dietary shift and thereafter it decreased at 6 and 12 months and remained at the same level at 48 months as at 12 months, which was ~25% higher when compared with the 0 month
In this investigation we were able to study long-term dietary effects since we followed 20 omnivores from before a dietary shift, during a 1 year lacto-vegetarian period and also 3 years after the end of the vegetarian diet period (48 months). Table III presents the faecal and urinary mutagenic activity before (0 months) and 3, 6 and 12 months after diet shift to a lacto-vegetarian diet and 3 years after the end of the vegetarian diet period (48 months).

Discussion

In this investigation we were able to study long-term dietary effects since we followed 20 omnivores from before a dietary shift, during a 1 year lacto-vegetarian period and also 3 years after the end of the lacto-vegetarian diet period (48 months). The use of frying (●) and eating raw vegetable meals (●) before (0 months), 3, 6 and 12 months after the dietary change and 3 years after the end of the vegetarian period when 17 of 20 had returned to a mixed diet (48 months) was used. The bacterial strain used was E.coli WP uvra.

Fig. 1. The use of frying (●) and eating raw vegetable meals (●) before (0 months), 3, 6 and 12 months after the dietary change and 3 years after the end of the vegetarian period when 17 of 20 had returned to a mixed diet (48 months). The numbers given in the food frequency index are mean points for each time period, where one point represents frying or eating a raw vegetable meal once a month (n = 20).

Fig. 2. The mutagenic activity in urine before the change in diet (0 months) and 3, 6 and 12 months after the change to a lacto-vegetarian diet. The bacterial strain used was E.coli WP uvra. The circles represent the sum of the positive wells, i.e. positive wells in the sample minus positive wells in the blank for each subject, and the horizontal lines represent the mean values for the groups. The filled circles represent the mixed diet (0 months) and the open circles the lacto-vegetarian diet (3, 6 and 12 months). There are 0, 2, 3 and 6 subjects with 0 positive wells at the above-mentioned time periods respectively (n = 20).

The use of frying decreased when changing to a lacto-vegetarian diet and increased slightly when changing back to a mixed diet 3 years after the end of the vegetarian period (Figure 1). The habit of eating a raw vegetable meal increased 3 months after the change to the vegetarian diet. This habit decreased somewhat during the rest of the follow-ups and remained at a higher level throughout the study compared with before the dietary shift.

The change from the mixed diet to the lacto-vegetarian diet led to a decrease in mutagenic activity in faeces when bacterial strain E.coli WP uvra was used. The mean numbers of positive wells were 52, 25, 35 and 25 for the various time periods respectively (Figure 2). The positive wells in urine for the vegetarian period (3, 6 and 12 months) were pooled and compared with the positive wells before the dietary shift (0 months). The mean value fell from 52 to 28 positive wells (P = 0.025, n = 20).

The change from the mixed diet to the lacto-vegetarian diet led to a decrease in mutagenic activity in faeces and the change back to a mixed diet led to an increase in the mutagenic activity when bacterial strain E.coli WP uvra was used. The mean numbers of positive wells were 9, 5, 6, 6 and 9 for the various time periods respectively (Figure 3). There was no statistically significant difference in faecal mutagenic activity between the two mixed diet periods (0 and 48 months) (P = 0.59). Therefore we were able to pool the mixed diet periods (0 and 48 months) and the lacto-vegetarian diet periods (3, 6 and 12 months) and compare the two diets. The mixed diet had a mean value of 8 positive wells and the vegetarian diet a mean value of 6 positive wells (P = 0.035, n = 19).

Mutagenic activity in faeces and urine tested with S.typhimurium TA98 gave very few positive wells for all time periods and no satisfactory dose–response curves were obtained. The same was also true for mutagenic activity in urine at 48 months tested with E.coli.

Discussion

In this investigation we were able to study long-term dietary effects since we followed 20 omnivores from before a dietary shift, during a 1 year lacto-vegetarian period and also 3 years after the end of the lacto-vegetarian diet period (48 months).
Experiments on humans have demonstrated that simultaneous consumption of parsley and fried salmon inhibits mutagenicity in urine compared with consumption of fried salmon alone (Ohyama et al., 1987).

There was also decreased intake of meat, fish and egg products, biscuits, coffee, tea and sweets during the vegetarian period. Meat and also protein intake has shown a positive correlation with colorectal cancer in several epidemiological studies (Gregor et al., 1969; Haenzel et al., 1973; Bjelke, 1974; Armstrong and Doll, 1975; Howell, 1975; Jain et al., 1980) and mutagen formation has been found to be related to the protein content of cooked food (Övervik and Gustafsson, 1990). One possible explanation for the increased risk for colon cancer associated with protein and meat relates to formation of mutagens during cooking. Meat, i.e. muscle protein, contains creatin(in)e, amino acids and sugar, which are important precursors of cooked food mutagens. Since a number of high protein, low creatin(in)e foods, such as organ meats, milk, cheese and beans, produce very low levels of mutagens compared with muscle meats, it is likely that the creatin(in)e level of foods is a limiting factor in mutagen production (Övervik and Gustafsson, 1990). The decrease in frying during the vegetarian period (Figure 1) might be as important as the changed intake of food components between the two diet periods, since food mutagens have been found mainly in cooked meat and seldom in foods of vegetarian origin. The ingestion of fried beef and pork may not only result in excretion of mutagens in faeces but also in urine (Baker et al., 1982; Dolara et al., 1984; Hayatsu et al., 1985; Sousa et al., 1985; Lindeskog et al., 1988). This has been shown in rodents, where mutagenic activity in urine can be detected after ingestion of food mutagens. Food mutagens have also been shown to be able to cause cancers in rats and mice at a number of sites (Övervik and Gustafsson, 1990).

Even though we found statistically significant differences between the mixed diet and the vegetarian diet in urinary and faecal mutagenic activity, we do not know the importance of these findings regarding the risk of developing colon cancer. It may be of crucial importance, since a small difference in mutagenic activity over a long period of time (years) may cause severe genetic damage. The repair system of the cell may not be able to keep up with continuous attack on its DNA, even at a low dose level.

On the other hand, the significance of the difference in mutagenic activity associated with the two diets may be minor, since the mutagenic activity may be counteracted by other factors. Dietary factors may, for instance, be of greater importance on the promotion side than on the initiation side, as indicated by the fact that mutagenic activity is far from zero on either diet. The diet may also promote defence mechanisms against cancer and the vegetarian diet may contain more so-called natural anti-carcinogens. Natural anti-carcinogens are naturally occurring agents which prevent activation of pre-carcinogens to carcinogens, interfere with the effect of carcinogens on target cells or suppress or delay multiplication of cancerous cells (Wattenberg, 1989). The vegetarian diet may, for instance, promote DNA repair mechanisms. In addition, dietary factors may influence the immune system; nutrient imbalance may for instance impair immunity (Beisel, 1984) and may thus influence early rejection of malignant cells (Wood and Watson, 1984).

To conclude, we have demonstrated that the mutagenic activity in faeces and urine was lower while volunteers were on a lacto-vegetarian diet compared with a mixed diet. There

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**Fig. 3.** The mutagenic activity in faeces before the change in diet (0 months), 3, 6 and 12 months after the change to a lacto-vegetarian diet and 3 years after the end of the study period when 17 of 20 volunteers had returned to a mixed diet (48 months). The bacterial strain used was *E. coli* WP2 uvrA. The circles represent the sum of positive wells, i.e. positive wells in the sample minus positive wells in the blank for each subject, and the horizontal lines represent the mean values for the groups. The filled circles represent the mixed diet before the dietary change (0 months) and the mainly mixed diet 3 years after the end of the vegetarian period (48 months). The open circles represent the lacto-vegetarian diet (3, 6 and 12 months). There are 5, 6, 7 and 1 subjects with 0 positive wells at the above-mentioned time periods respectively. Due to insufficient faecal material, n = 17 for 0 and 3 months, n = 18 for 6 and 12 months and n = 19 for 48 months.

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One drawback with this study is that it was designed and performed before 1989, when Bosworth and Venitt (1989) published an article dealing with the heterogeneity of bacterial fluctuation test data and the effects of auxotrophic growth enhancement. The interpretations of our data should therefore be made with caution and the study should be repeated taking into account the recommendations suggested by Bosworth and Venitt.

This study demonstrates increased intake of fruits, berries, vegetables, cereals and dairy products. These food items contain compounds that may contribute to an explanation of our results. The increased fibre intake during the vegetarian period caused an increase in faecal weight, mainly due to an increased water content in faeces and thereby dilution of faecal mutagenic compounds. Binding of mutagens by fibre may also contribute to this decrease (Lindeskog et al., 1988). Furthermore, vitamin C has shown an inverse relationship to faecal mutagenic activity in a number of studies (Bruce and Dion, 1980; Dion et al., 1982). Mechanistically this effect is not clear and the inverse relationship may be explained by other food components in vitamin C-rich foods. Several compounds in foods and plants have an inhibitory effect on mutagens/carcinogens. For instance, certain plant phenols and indoles inhibit neoplasia induced by polycyclic aromatic hydrocarbons (Wattenberg, 1979). Ellagic acid, abundant in grapes and nuts, reduces mutagenicity of benzo[a]pyrene (Wood, et al., 1982). Also, a positive correlation between chlorophyll content of certain vegetables and their antimutagenic activity against 3-methylcholanthrene has been found (Lai et al., 1980). Experiments on humans have demonstrated that simultaneous
are a multitude of food components that may explain these changes. However, the importance of the changes in faecal and urinary mutagenic activity with regard to cancer risk is still not known. Nevertheless, the search for genotoxic activity in human faeces and urine may provide valuable clues to the aetiology of cancers and the knowledge gained may eventually lead to means for reducing the incidence of this disease.

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