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Viewpoint

Health promoting compounds in vegetables and fruits: A systematic approach for identifying plant components with impact on human health

K. Brandt[a,*], L.P. Christensen[b], J. Hansen-Møller[d], S.L. Hansen[b], J. Haraldsdottir[c], L. Jespersen[c], S. Purup[d], A. Kharazmi[e], V. Barkholt[f], H. Frøkiær[f] and M. Kobæk-Larsen[g]

[a]University of Newcastle upon Tyne, School of Agriculture, Food and Rural Development, King George VI Building, Newcastle upon Tyne, NE1 7RU, UK (fax: +44-1661-831-006; e-mail: kirsten.brandt@ncl.ac.uk)

[b]Danish Institute of Agricultural Sciences, Department of Food Science, Kirstinebjergvej 10, DK-5792 Aarslev, Denmark

[c]Royal Veterinary and Agricultural University, Department of Human Nutrition, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

[d]Danish Institute of Agricultural Sciences, Department of Animal Nutrition & Physiology, Research Centre Foulum, DK-8830 Tjele, Denmark

[e]Copenhagen University Hospital, Department of Clinical Microbiology, Tagensvej 20, 2200 København N, Denmark

[f]Technical University of Denmark, Biocentrum, 2800 Lyngby, Denmark

[g]University of Southern Denmark, Biomedical Laboratory, Winsløwparken 23, 5000 Odense C, Denmark

* Corresponding author.

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Vegetables contain unknown compounds with important health promoting effect. The described project defined and tested a two-step screening procedure for identification of such compounds. Step 1 is initial screening according to three criteria: 1.1, chemically reactive functional groups; 1.2, toxicity at high concentrations or other bioactivity; and 1.3, presence in healthy foods. Step 2 is testing for minimum criteria defining health-promoting compounds: 2.1, positive or biphasic ("hormesis") responses in bioassay; 2.2, human tissue concentrations corresponding to beneficial effects in bioassay; and 2.3, possibility to control content in food. Falcarinol from carrots fulfilled all 6 criteria and subsequently showed anticancer effect in rats.

Introduction

Optimisation of composition of plant-derived food would be a very cost-effective method for disease prevention, since diet-induced health improvements would not carry any added costs for the health sector (Gundgaard, Nielsen, Olsen, & Sørensen, 2003). If improvements can be obtained with existing or slightly adapted food technology, the production costs will be similar.

Many epidemiological studies show negative correlations between the intake of vegetables and fruits and the incidence of several important diseases, including cancer

and atherosclerosis (Trichopoulou, Naska, Antoniou, Friel, Trygg, & Turrini, 2003; Kris-Etherton, Etherton, Carlson, & Gardner, 2002; Maynard, Gunnell, Emmett, Frankel, & Smith, 2003; Gundgaard *et al*., 2003). Vegetables and fruits are known to contain components with several types of health promoting actions (as vitamins, as essential minerals, as antioxidants and as prebiotics (fibres)) and most of these have been evaluated in intervention studies. In general, health benefits from supplementation have been proved only for groups that had particularly low intake of these compounds, e.g. due to malnutrition, while supplementations at high levels generally provided only minor additional improvements, and in some cases even showed adverse effects, as is the case for β-carotene (Omenn *et al*., 1996). However, the data from epidemiological studies shows sufficient linearity across the range to allow calculations of the benefits of substantial increases in the intake of fruits and vegetables to 400 g per day (van't Veer, Jansen, Klerk, & Kok, 2000) or even higher (Gundgaard *et al*., 2003). This should be compared with the lower levels (approx. 200 g per day) needed to ensure against deficiencies of the known beneficial compounds in the absence of supplementation or other dietary sources (Ali & Tsou, 2002). Those levels are lower than the average intake in European countries (Agudo *et al*., 2002). In addition, a few compounds are known or suspected to benefit health by other, less understood mechanisms, such as protection against cardiovascular disease by sulfoxides and/or flavonoids from Alliums (Griffiths, Trueman, Crowther, Thomas, & Smith, 2002) or anticancer effects of glucosinolates and their derivatives (Lund, 2003; Thomalley, 2002) from Brassicas (Kassie *et al*., 2003; Smith, Mithen, & Johnson, 2003). However, the epidemiological correlations are not confined to these two types of vegetables.

While the consensus is that most people should eat more vegetables and fruit, rather than pills supplying adequate amounts of the relevant nutrients, it is not known why this is in fact better, nor how to advice people who cannot or will not eat the full selection in large amounts. As long as the compounds that make the greatest difference for health have not been identified, knowledge of the fate of plant constituents during cooking and storage cannot be used to conclude to what extent raw plant foods are better or worse than cooked ones or if a storage method increases or decreases the value for health. Similarly, the knowledge on biodiversity and plant composition does not tell us to what extent certain species, cultivars or cultivation conditions are important for this value. So while very specific and practically useful dietary recommendations are provided for groups of people who are at risk for malnutrition (Darmon, Ferguson, & Briend, 2002), the advice to the affluent populations is to use a variety of species and preparation methods for the vegetables and fruit (Trichopoulou *et al*., 2003; Kris-Etherton *et al*., 2002). At present the primary background for this recommendation is our ignorance, diversity maximises the random chance of obtaining sufficient amounts of the crucial, largely unknown compounds, as long as we do not know which foods are the best sources (Trichopoulou *et al*., 2003). Once we have identified these compounds and sources, we will probably still recommend diversity, but then the advice will be based on knowledge, and it will become possible to adjust and optimise the recommendations for people with special needs or preferences.

Specifically, the present information shows that compounds with already investigated beneficial properties, for example antioxidants, either have additional or unknown benefits and properties, or foods from plants simply contain other health promoting compounds with unknown effects, that have until now been overlooked. Many studies, including several EU-projects, such as QLK1-CT-2001-01080, QLK1-CT-1999-00830, QLK1-CT-1999-00505, QLK1-CT-1999-00498, QLK1-CT-1999-00124, BMH4960726 and COST 916, aim at investigating new properties and/or interactions of known compounds. In contrast, few studies focus on the identification of new types of health promoting compounds from vegetables and fruits. The multidisciplinary project "Health promoting compounds from vegetables" described in the present paper was designed to initiate a systematic approach for screening for novel compounds from edible plants that are likely to have important effects on health. The work took place between January 2000 and October 2003, and the present paper summarises the concepts, findings and perspectives of this project.

Concept and content

To ensure efficient use of resources on the systematic screening for health promoting compounds from edible plants, the 3-step protocol below was defined.

1. Identification of candidate compounds based on existing knowledge of their properties, by checking to what extent they meet a set of criteria that is designed to exclude compounds with minor effects.
2. Initial experimental investigation of the most promising candidates according to a second set of predefined characteristics, which are generally not known for less-studied compounds.
3. Further investigations of specific properties and possible modes of action of the compounds selected from the second step.

Within the project two objectives were defined:

(i) to establish a network of scientists to discuss and endorse the criteria for selection and list some relevant candidates according to the step 1

criteria, and to establish an expertise base to perform initial and more advanced investigations of compounds from fruits and vegetables with potential for disease prevention.

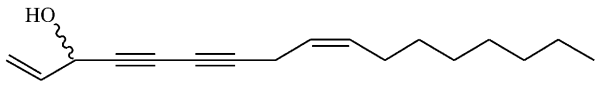
(ii) to test the concept by performing the initial investigation (step 2) of the compound falcarinol (Fig. 1) from carrots. If falcarinol failed one of the criteria of this step, studies would be initiated on another compound from the list of candidates.

The criteria used for selection and ranking of candidates in step 1 were:

1.1 Presence of chemically reactive functional groups or other chemical properties indicating likely interaction with cell components.

1.2 Known effect on some physiological functions in humans, such as the immune system (allergenic) or the kidneys (diuretic), toxicity at high concentrations or other known bioactivity including antimicrobial and antinutritional effects.

1.3 Presence in foods which are widely consumed, preferably foods epidemiologically linked with decreased risk of disease, indicating absence of direct toxicity at the concentrations normally found in food and indicating good prospects that new knowledge can and will be utilised to improve diets.



Falcarinol

Figure 1. Chemical structure of falcarinol.

Toxicity at high concentrations was considered to be a positive aspect because most new health promoting compounds would be likely to show biphasic biological effects (hormesis) (Calabrese & Baldwin, 1998; see also Fig. 2). Previous research on food and health has mainly been focused on either beneficial effects of nutrients, which can be identified by their ability to alleviate malnutrition symptoms (Pomerleau, McKee, Lobstein, & Knai, 2003) or on harmful effects of toxicants, which are identified by their detrimental effects at very high concentrations (Essers *et al.*, 1998). This study aims to search for compounds that have positive effects at low concentrations as normally found in food, and for this purpose looking for toxicants in health-promoting food could be an efficient way to distinguish bioactive compounds from those with no effect at low levels. Also, we considered the possibility that the categorisation of a compound as toxicant could have deterred other scientists from initiating studies of beneficial effects of these compounds, increasing the chance for significant new discoveries in this particular group of compounds. Glucosinolates and isothiocyanates are an example of a group of compounds established as anticancer agents that were originally best known as toxicants (Lund, 2003).

The criteria used for testing in step 2 were:

2.1 Dose-dependent biological response in one or more relevant bioassays, which indicates some positive effect on health (not necessarily specific for a particular mode of action), and which also allows recording of toxic effects at super-optimal concentrations.

2.2 That after ingestion of a relevant food, concentration in a human tissue reaches levels corresponding to the range that elicits the

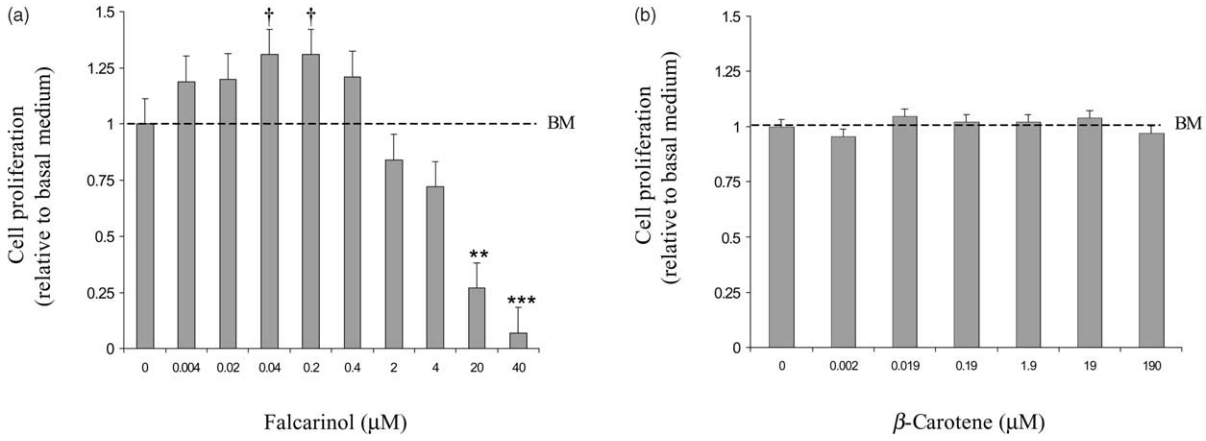


Figure 2. Effects of increasing concentrations of falcarinol (a) and β-carotene (b) on proliferation, measured by incorporation of [methyl-^{3}H]thymidine into mammary epithelial cells prepared from prepubertal Frisian heifers and grown in 3-dimensional collagen gels. Values are least square means ± SEM obtained from cultures with triplicate samples and presented as relative to proliferation obtained in basal medium. Cell proliferation values significantly different from those obtained in BM are indicated: †$P < 0.09$, **$P < 0.01$, *** $P < 0.001$. (Redrawn from Hansen *et al.*, 2003)

potentially positive effects shown in the bioassay of criterion 2.1. This would indicate that the bioavailability is sufficiently high to ensure that the effects tested in the bioassay are physiologically relevant, but not so high that toxic effects are likely to predominate.

2.3 A variable occurrence in food raw materials (e.g. a response to common processing methods and/or significant differences among plant cultivars), which show that the content in foods can be controlled and optimised using existing or only slightly adapted technology.

If information in support of one or more step 2 criteria is available in advance, this should also be taken into account by increasing the ranking of compounds for selection at step 1.

Compounds selected in step 2 by fulfilling all 3 criteria should then be subjected to further studies, step 3. The investigations here should be tailored to the specific compound, aiming to provide the additional knowledge that must be obtained in order to quantify the compound's role in preventing disease, and eventually to improve the impact of food on health. In most cases the following types of studies would be relevant in step 3:

- Long-term animal studies, including toxicological investigations.
- Investigations of basic mechanisms of action.
- Thorough investigations of bioavailability, including distribution in different tissues.
- Epidemiological investigations, which may include analysis of blood samples from existing cohort studies.
- Basic investigations of the role of the compound in the plant.
- Development of diets or foods with optimised levels of the compound.

Results

Selection of candidate compounds (step 1)

Of the thousands of secondary metabolites of vegetables, most types were dismissed summarily due to low chemical reactivity (e.g. surface waxes), low and/or unknown occurrence in food (e.g. naphtalene glycosides) or little data on relevant biological activities (e.g. many aromatic compounds). Based on such considerations and the range of expertise of the project participants, some groups of compounds were assessed in more detail according to the criteria of step 1, as listed in overview in Table 1. For those that fulfilled all three criteria, and were not already under intensive investigation in other contexts, the criteria were evaluated quantitatively, in order to select one compound for further studies within the project.

Criterion 1.1 (reactive chemical groups)

Polyacetylenes contain triple bonds that can stabilise radicals and/or carbocations formed at adjacent positions in the molecules, and falcarinol can form an extremely stable carbocation. The α-methylene-γ-butyrolactone entity of sesquiterpene lactones is a powerful electrophile. Both types of compounds readily react with amino groups in protein and other nucleophilic sites of biomolecules under conditions occurring in biological materials (Christensen & Larsen, 2003).

The carbon skeleton of the coumarins forms a conjugated system with some ability to stabilise radicals, although the tendency to engage in formation of chemical bonds with other molecules is substantially less than for polyacetylenes and sesquiterpene lactones.

The aglycons of glycoalkaloids have chemical properties that allow them to enter membranes and

Table 1. Examples of candidate compounds for screening and the assessments, ranked in descending order according to how well they fulfil the criteria in step 1

Name	Food source	Assessment
Organosulphur compounds (glucosinolates, ACSOs and their degradation products)	Brassicas, onion	Meet all the criteria for step 1, are generally recognised as potentially health promoting compounds and presently under investigation in other projects.
Polyacetylenes, including falcarinol	Carrot, parsnip, green tomato	Meet all the criteria for step 1, selected for studies to test for step 2 in the project.
Sesquiterpene lactones	Chicory and lettuce	Meet all the criteria for step 1, recommended to test for step 2.
Phenolic and carotenoid antioxidants	Many different vegetables and fruits	Meet all the criteria for step 1, and have been extensively studied, however, the criterion for step 2.2 (human tissue concentrations corresponding to *in vitro* effects) is not clearly met.
Glycoalkaloids	Potato, tomato	Meet all the criteria for step 1, recommended to test for step 2.
Coumarins and isocoumarins	Carrot, potato, chicory etc.	Meet all the criteria for step 1, recommended to test for step 2.
Stevioside	Stevia rebaudiana *tea (from dried leaves)*	Miss criterion 1.3. This food is not widely consumed in Europe.

affect their structure and function, and thus fulfil criterion 1, although these compounds are not directly chemically reactive (Plhak & Sporns, 1997).

So of these four types of compounds, falcarinol and sesquiterpene lactones have the most pronounced chemical reactivity, while the coumarins and glykoalkaloids were at a lower level for this criterion.

Criterion 1.2 (physiological effect, bioactivity)

For this characteristic, there was a substantial body of experimental data for each of the four types of compounds, generally focusing on different forms of biological activity, for example membrane disruption and cholinesterase inhibition for glycoalkaloids (Plhak & Sporns, 1997), effects on P450 enzymes for coumarins (Clifford, 2000) and inhibition of transcription factors for sesquiterpene lactones (Wong & Menendez, 1999). Due to this and the outcome for the two other criteria it was not attempted to rank the four types of compounds according to this criterion, and only the data on falcarinol are presented here.

Biological effects *in vivo*: Falcarinol can act as a hapten to cause allergic dermatitis due to skin exposure (Hansen & Boll, 1986). It kills brine shrimp at relatively low concentrations (Cunsolo, Ruberto, Amico, & Piattelli, 1993) and also prevents the development of several fungal diseases, including liquorice rot in carrots (Olsson & Svensson, 1996).

In vitro effects: Several reports on effects of falcarinol on cancer cells are available (reviewed in Brandt & Christensen, 2000), one of these showed cytotoxic effects on human gastric adenocarcinoma cells (Matsunaga, Katano, Yamamoto, Fujito, Mori, & Takata, 1990) at low falcarinol concentrations (ED_{50} of 0.11 μM), while non-cancer fibroblasts survived at concentrations 20 times higher than this.

Criterion 1.3 (presence in food plants, epidemiology)

Many studies have shown that a high content of natural β-carotene in blood is correlated with a low incidence of several types of cancer, while intervention studies have shown that supplementation with β-carotene does not protect against development of this disease (e.g. Omenn *et al*., 1996; Greenberg *et al*., 1996; and references herein). In most European countries and North America more than 50% of the β-carotene intake is provided by carrots (O'Neill *et al*., 2001). Carrots are the only major food items that are known to contain falcarinol, thus probably the major dietary source of this compound. In these regions carrot consumption is better correlated with the intake of α-carotene than with the intake of β-carotene (O'Neill *et al*., 2001). Three out of four studies found stronger negative correlations of lung cancer with intake of α-carotene than β-carotene (Wright, Mayne, Swanson, Sinha, & Alavanja, 2003; Michaud *et al*., 2000; Knekt, Järvinen, Teppo, Aromaa, & Seppänen, 1999; Ziegler *et al*., 1996). These reports all implicated carrots, but findings could be related to compounds other than carotenoids. Falcarinol is also found in *Panax ginseng*, which is best known as a medicinal plant, but in Europe also has some use in food supplement preparations and teas. In the fresh plant material falcarinol occurs at a concentration of 700 mg kg^{-1} dry weight (Kitagawa, Yoshikawa, Hayashi, & Taniyama, 1983), which is approx. 5 times higher than in carrots (Hansen, Purup, & Christensen, 2003).

In contrast, only few epidemiological studies link potatoes (the primary dietary source of glycoalkaloids) with low risk of particular diseases (Tavani, Bosetti, Negri, Augustin, Jenkins, & La Vecchia, 2003), possibly due to confounding with other food and lifestyle factors (Ursin, Ziegler, Subar, Graubard, Haile, & Hoover, 1993). For lettuce/chicory (the primary dietary source of sesquiterpene lactones) most epidemiological studies did not distinguish between these and other green leafy vegetables such as rucola, spinach and Chinese cabbage (Williams, Wareham, Cox, Byrne, Hales, & Day 1999; Wright *et al*., 2003; Knekt *et al*., 1999; Ziegler *et al*., 1996), which makes it difficult to define a precise link with intake, in particular since some of these species contain glucosinolates. Coumarins are widely distributed in several types of vegetables, including carrots, potatoes and chicory, and there is only scattered information on their actual concentrations in foods (Clifford, 2000), so it is even more difficult to link this type of compound with a health promoting effect based on existing epidemiological data. The differences between the compound groups thus primarily relate to their distribution among food items, and most likely also reflect that high interest in β-carotene in earlier studies has led to a greater volume of data relating to carrots. So while the data on vegetables in general (van't Veer *et al*., 2000) imply that every compound present in substantial amounts in any widely consumed vegetable can be considered to fulfil this criterion, they do not rule out substantial health promoting effects of those compounds for which few specific data are available. Still, for the purpose of ranking, for this criterion it must be concluded that the link between falcarinol (carrot intake) and cancer is the best documented one.

Information in support of one or more step 2 criteria (bioavailability).

Saita *et al*. (Saita, Matsunaga, Yamamoto, Nagumo, Fujito, Mori, & Katano 1994) reported that panaxytriol, a polyacetylene closely related to falcarinol, could be measured in rat plasma after oral intake, indicating that an attempt to measure bioavailability of falcarinol in humans was likely to succeed.

It was thus concluded that with the data available in 2001, compared with the other compounds listed in Table 1, and excluding the already recognised health promoting glycosinolates and onion sulfoxides, falcarinol

was the compound that best fulfilled the criteria for step 1, and thus should be subjected to investigations of the criteria for step 2.

Initial investigations of falcarinol (step 2)

Criterion 2.1 (Dose-dependent biological response)

The bioassay used was proliferation of cultured primary bovine mammary epithelial cells, measured as incorporation of [methyl-^{3}H]thymidine (Hansen *et al.*, 2003). A broad range of concentrations of falcarinol was evaluated, and for comparison the effects of β-carotene were also measured. For falcarinol the assay showed a typical biophasic pattern indicative of hormesis (Fig. 2), while no significant effect was measured for β-carotene (Fig. 2). The results indicated a positive response between 0.002 and 0.4 μM of falcarinol. Below 0.002 μM no effect was detected, from 4 μM inhibition of proliferation was apparent, possibly as a result of toxic effects. The value at 2 μM appears to represent a range influenced by both stimulatory and toxic effects.

Criterion 2.2 (concentration in human tissue)

The published rat study used an ELISA method for analysis of panaxytriol in rat plasma (Saita *et al.*, 1994), and in the project it was first attempted to reproduce this method for falcarinol. However, even though anti-falcarinol antibodies were obtained by immunising rabbits with conjugated falcarinol according to a method described earlier (Saita, Katano, Matsunaga Yamamoto, Fujito, & Mori, 1993; Saita *et al.*, 1994), the affinity was too low to provide good measurements at the relevant concentrations. Consequently, an LC-MS method was developed which allowed measurement of falcarinol concentrations down to 0.001 μM (Hansen-Møller *et al.*, 2002). In a preliminary test with two subjects an intake of 800 ml of carrot juice containing 110 μmol falcarinol (28 mg) resulted in human plasma concentrations of up to 0.06 μM falcarinol (Hansen-Møller *et al.*, 2002). This preliminary study was followed up by a more detailed study (Haraldsdóttir, Jespersen, Hansen-Møller, Hansen, Christensen, & Brandt, 2002), where fourteen males received doses of 300, 600 and 900 ml of another batch of carrot juice for breakfast, containing 16, 33 and 49 μmol falcarinol, respectively. The level of falcarinol was measured in plasma samples collected at 10 intervals during an 8-h period starting just before breakfast. All three doses resulted in rapid increases in plasma falcarinol, within half an hour after the meal. The values peaked after 2 h with concentrations between 0.004 and 0.01 μM plasma for each dose when averaged across subjects, coming almost down to baseline at 8 h (Fig. 3).

Criterion 2.3 (Opportunities for optimising content in food)

The level of falcarinol in fresh carrot tissue varied between 22 and 56 μM (5.5–13.7 mg kg^{-1}) (Kidmose, Hansen, Nørbæk, Christensen, & Edelenbos, 2003; Kidmose, Hansen, Christensen, Edelenbos, & Nørbæk, unpublished data) among 6 carrot cultivars of the Nantes type, orange cylindrical carrots normally sold as fresh carrots in Northern Europe. It is likely that other carrot types will differ even more, since the genetic variation within the Nantes type is relatively low (St. Pierre, & Bayer, 1991).

The falcarinol content of whole carrots was reduced by 37% during 4 months of storage at 1°C (Hansen *et al.*, 2003). Blanching of 1 cm^3 cubes for 75 s at 90°C caused a similar reduction, but the content was stable during subsequent storage at −24°C. Boiling of 2.5 cm slices for 12 min reduced the falcarinol content by 50% and most of the loss occurred during the first 3 min (Hansen *et al.*, 2003).

Further studies (step 3)

The first step of such further studies has been initiated, in the form of an *in vivo* test for anticancer effect (Kobæk-Larsen, Christensen, Vach, Ritskes-Hoitinga, & Brandt, unpublished work). Using an established rat model for colon cancer, induction by injections of azoxymethane in the inbred rat strain BDIX/OrlIco (Kobæk-Larsen *et al.*, 2002), it was investigated whether intake of falcarinol would affect the occurrence or development of malignancies in this system. Three groups of 10 rats were fed the standard rat feed Atromin® supplemented with either 10% (w/w) freeze dried carrots containing 0.14 μM falcarinol (carrot treatment), 10% maize starch and 0.14 μM falcarinol isolated from carrots (falcarinol treatment), or 10% maize starch (control). Two weeks after the start of the dietary treatments, eight rats in each treatment received the first of 4 injections of the carcinogen azoxymethane (AOM)

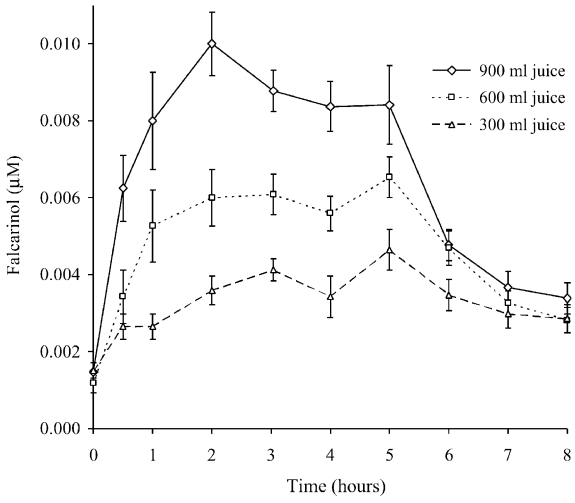


Figure 3. Concentration of falcarinol in plasma of 14 volunteers as a function of time after ingestion of a breakfast meal with 300, 600 and 900 ml carrot juice, containing 16, 33 and 49 μmol falcarinol, respectively. Means ± SEM. (Redrawn from Haraldsdóttir *et al.*, 2002)

(Kobæk-Larsen *et al*., 2002) and 18 weeks after the first AOM injection, the rats were killed and the colon was examined for tumours and their microscopic precursors, aberrant crypt foci (ACF) (McLellan & Bird, 1998). The body weight development was identical in all three groups. The carrot and falcarinol treatments showed a significant ($P=0.028$) tendency to reduced numbers of (pre)cancerous lesions with increasing size of lesion (Fig. 4), when the four size classes were scored from 1 to 4 with increasing lesion size and the normalised numbers of ACFs/tumors were modelled in the two treatment groups as a function of the parameter score. The correlation among the size classes within each individual was taken into account by using robust variance estimates. This suggests that the protective effect of carrot can be explained to a high degree by its content of falcarinol. The overall potency and the interaction with lesion size were similar to those of the glucosinolate sinigrin (Smith, Lund, & Johnson, 1998) and Brassica vegetables (Kassie *et al*., 2003; Smith *et al*., 2003) tested in similar models.

Discussion

Implications of the data on falcarinol

The range of concentrations that resulted in stimulatory effects in the bioassay (Fig. 2) was of the same magnitude as the plasma concentrations (Fig. 3) found after intake of carrot juice. This strongly indicates that ingestion of falcarinol-containing food is likely to affect cells in the human body. Of course these results do not give any information whether such an effect is beneficial or detrimental to health, only that an impact will occur. However, when our results are combined with the epidemiological investigations cited earlier, which consistently show negative correlations between carrot consumption and cancer, and the preliminary data on rat colon cancer (Fig. 4), they strongly indicate that falcarinol does something to the body that reduces cancer incidence. Together with the absence of benefits of β-carotene in intervention studies (Omenn *et al*., 1996; Greenberg *et al*., 1996) and of effect of β-carotene in the *in vitro* assay (Fig. 2), this leads to the conclusion that some or all of the benefits previously assumed to be due to intake of carrot carotenes, may in fact be caused by falcarinol.

The above mentioned data on effects of genotypes, processing and storage show that the level of falcarinol in foods can easily be increased, using methods that can be handled within the existing expertise and facilities of many companies operating at different levels of the food chain. So if a beneficial effect of falcarinol is confirmed in further scientific investigations, and toxicological studies have defined which levels are safe in food, industry can quickly raise the level of falcarinol substantially in carrots and in foods based on this vegetable. One of the genes in the biosynthetic pathway to polyacetylenes has already been cloned (Cahoon, Schnurr, Huffman, & Minto, 2003), so it may become possible to introduce production of falcarinol also in crops where it does not normally occur, if a need for this arises. On the other hand, due to the earlier described allergenic and toxic potentials of the compound, it would probably be preferable to keep it confined to carrots and the few other plant species where it occurs naturally (Brandt & Christensen, 2000), to enable consumers to control the exposure according to individual sensitivity.

The exact nature and magnitude of a cancer preventive effect of falcarinol is still unknown, nor is it clear if other compounds with important biological effects are present in carrots, and there are only vague

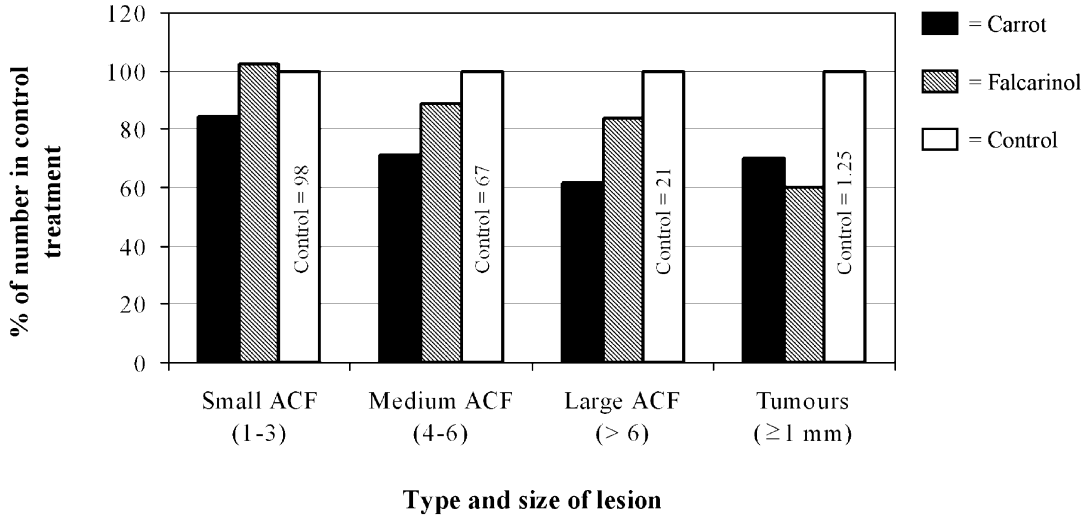


Figure 4. Effect of treatments with carrot or falcarinol on the numbers per animal of four types of (pre)cancerous lesions in rat colons, each size class representing increasingly advanced steps on the progression towards cancer. The size of aberrant crypt foci (ACF) is measured as the number of crypts found on a corresponding area of normal colon tissue. The smallest tumours correspond to an ACF size of approx. 20. (Redrawn from Kobæk-Larsen *et al*., unpublished work)

clues as to what mechanisms may be operating, regarding both beneficial and toxic effects. These subjects should now be in focus for the next steps of the scientific part of this line of investigation.

Applicability of the screening approach

The systematic use of the screening criteria in step 1, based on literature data, made it possible to select a candidate compound, which then turned out to possess all of the three the predefined characteristics that were tested experimentally in step 2 during the project. It must be emphasised that while many compounds are known to fulfil the step 1 criteria, the step 2 criteria are quite strict, in particular 2.2 (that concentration in a human tissue after a normal food intake reaches a level which is effective in a relevant bioassay). The formulation of this criterion was inspired by the methods used for identifying potent pharmaceuticals (Garrett & Workman, 1999), and while corresponding data are available for several pharmaceuticals (Krejsa *et al.*, 2003), the authors are not aware of food non-nutrients other than falcarinol that has been reported to fulfil this criterion. Generally it has not yet been shown explicitly that the ranges that are effective in *in vitro* tests correspond to the concentrations measured in human plasma or other relevant tissues, even for thoroughly studied bioactive plant compounds, such as phenolic antioxidants (Astley, 2003) and glucosinolates (Lund, 2003).

In some cases other approaches can be more useful. For example, where a plant food provides specific health benefits, which do not correspond with the known or probable effects of any of the known compounds it contains, bioassay-guided fractionation should be considered (Brandt, Christensen, Larsen, Kharazmi, Purup, & Christensen, 2001). It has for example been used to identify an anti-inflammatory compound from a preparation (Hyben Vital®, Hyben Vital International ApS, Tullebølle, Denmark) of dried rose-hips, the fruits of the wild rose *Rosa canina* (Larsen, Kharazmi, Christensen, & Brøgger Christensen, 2003). However, this technique requires that an efficient bioassay for the specific effect is available, and that there is good reason to believe that the bioassay really measures the same effect as found in human subjects.

Toxicants and hormesis

Since criterion 1.2 of the screening procedure was "toxicity at high concentrations", it is to be expected that many compounds identified using the described procedure will also be known as food toxicants, as is the case for falcarinol. Inherent food plant toxicants are defined as "plant constituents which might give rise to adverse effects in humans when the plant or plant products are ingested." (Essers *et al.*, 1998), and a list of these compounds was produced by the EU-project NETTOX (Gry *et al.*, 1998). It has for some time been recognised that some toxicants can have beneficial effects (Essers *et al.*, 1998), but also considered "prudent to consider the adverse, rather than the possible health promoting effects, for risk assessment" for non-essential substances, and testing of a compound for beneficial effects was not included in the list of disciplines involved in risk assessment on intrinsic plant food toxicants (Essers *et al.*, 1998).

However, it has recently been demonstrated that the majority of toxic compounds exhibit hormesis, whenever test designs are used that allows this to be detected (Calabrese & Baldwin, 2003), exactly in line with the concept and findings of the present study. When hormesis is taken into consideration, the determination of the beneficial intake levels becomes a crucial aspect of the risk assessment (Calabrese & Baldwin, 2002). In fact, systematic investigations of beneficial effects of the many inherent toxicants that fulfil the screening criteria defined in the presently described project are necessary as a prerequisite for any comprehensive risk assessment of these substances. As formulated by Essers *et al.* (1998): "the margin between the levels which result in positive or negative health effects, respectively, as well as intake in the population, should be established before intake levels are regulated". Examples of compounds that should receive particularly high priority from this point of view, are those where the content in food is already regulated or considered for regulation, such as potato glycoalkaloids and several natural flavour components (Essers *et al.*, 1998) and acrylamide (Mucci, Dickman, Steineck, Adami, & Augustsson, 2003), all of which fulfil all the step 1 criteria of the procedure described in the present paper.

Conclusion and future trends

Conclusion

The project has provided a structured approach for selection of bioactive compounds in plant foods, which are likely to have important impact on health, and where there are good prospects that this knowledge will lead to food with improved nutritional quality. The project demonstrated that the described approach could be used to identify a compound, falcarinol, that was subsequently shown to reduce cancer development in a rat model. The approach should be used to assess additional compounds, and the ones which fulfil the defined criteria should be subjected to further studies regarding their beneficial effects on human health.

Future trends

The approach described here can primarily be used for two purposes: One is to identify compounds responsible for health promoting properties of foods, and thus allow targeted improvement of the composition of the food, and maybe also discovery of compounds

with therapeutic potential. The other purpose is to support the risk assessment of compounds known or suspected for toxic effects, where a similar approach can be used to indicate the levels of intake where beneficial effects should be investigated, in order to ensure that a regulation does not inadvertently reduce the dietary intake below the optimal level for health. Both purposes are based on the concept that it is indeed possible to model the effect of a food as a function of its constituents.

Improved knowledge of which compounds and concentrations in food are beneficial for health will be crucial for future systematic efforts to improve food quality. From organic agriculture to genetically modified crops, and from ethnic specialities to hospital food, a primary impediment to improvements of food quality is the difficulty of finding measures that distinguish healthy foods from the not so healthy versions. As far as possible, the optimal ranges for the key food components should be defined, as a tool to develop and ensure foods with improved effects on health.

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References

Agudo, A., Slimani, N., Ocke, M. C., Naska, A., Miller, A. B., Kroke, A., Bamia, C., Karalis, D., Vineis, P., Palli, D., Bueno-de-Mesquita, H. B., Peeters, P. H. M., Engeset, D., Hjartaker, A., Navarro, C., Garcia, C. M., Wallstrom, P., Zhang, J. X., Welch, A. A., Spencer, E., Stripp, C., Overvad, K., Clavel-Chapelon, F., Casagrande, C., & Riboli, E. (2002). Consumption of vegetables, fruit and other plant foods in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts from 10 European countries. *Public Health Nutrition*, *5*, 1179–1196.

Ali, M., & Tsou, S. (2000). The integrated research approach of the Asian Vegetable Research and Development Center (AVRDC) to enhance micronutrient bioavailability. *Food and Nutrition Bulletin*, *21*, 472–481.

Astley, S. B. (2003). Dietary antioxidants — past, present and future?. *Trends in Food Science & Technology*, *14*, 93–98.

Brandt, K., & Christensen, L. P. (2000). Vegetables as nutraceuticals — falcarinol in carrots and other root crops. In I. T. Johnson, & G. R. Fenwick (Eds.), *Dietary anticarcinogens and antimutagens. chemical and biological aspects* (pp. 386–391). UK: Royal Society of Chemistry.

Brandt, K., Christensen, L. P., Larsen, E., Kharazmi, A., Purup, S., & Christensen, S. B. (2001). Using traditional medicine knowledge as a lead to selection of potentially health promoting compounds in food plants. In W. Pfannhauser, G. R. Fenwick, & S. Khokhar (Eds.), *Biologically-active phytochemicals in food. analysis, metabolism, bioavailability and function* (pp. 584–588). Cambridge, UK: The Royal Society of Chemistry.

Cahoon, E. B., Schnurr, J. A., Huffman, E. A., & Minto, R. E. (2003). Fungal responsive fatty acid acetylenases occur widely in evolutionarily distant plant families. *Plant Journal*, *34*, 671–683.

Calabrese, E. J., & Baldwin, L. A. (1998). Hormesis as a biological hypothesis. *Environmental Health Perspectives*, *106*, 357–362.

Calabrese, E. J., & Baldwin, L. A. (2002). Applications of hormesis in toxicology, risk assessment and chemotherapeutics. *Trends in Pharmacological Sciences*, *23*, 331–337.

Calabrese, E. J., & Baldwin, L. A. (2003). The hormetic dose-response model is more common than the threshold model in toxicology. *Toxicological Sciences*, *71*, 246–250.

Christensen, L. P., & Larsen, E. (2003). Secondary products: Allergens. In B. Thomas, D. Murphy, & B. Murray (Eds.), *Encyclopedia of Applied Plant Sciences, Vol 3* (pp. 1159–1174). London: Academic Press.

Clifford, M. N. (2000). Miscellaneous phenols in foods and beverages — nature, occurrence and dietary burden. *Journal of the Science of Food and Agriculture*, *80*, 1126–1137.

Cunsolo, F., Ruberto, G., Amico, V., & Piattelli, M. (1993). Bioactive metabolites from Sicilian marine fennel, *Crithmum maritimum*. *Journal of Natural Products*, *56*, 1598–1600.

Darmon, N., Ferguson, E., & Briend, A. (2002). Linear and nonlinear programming to optimize the nutrient density of a population's diet: an example based on diets of preschool children in rural Malawi. *American Journal of Clinical Nutrition*, *75*, 245–253.

Essers, A. J. A., Alink, G. M., Speijers, G. J. A., Alexander, J., Bouwmeister, P. J., van den Brandt, P. A., Ciere, S., Gry, J., Herrman, J., Kuiper, H. A., Mortby, E., Renwick, A. G., Shrimpton, D. H., Vainio, H., Vittozzi, L., & Koeman, J. H. (1998). Food plant toxicants and safety — Risk assessment and regulation of inherent toxicants in plant foods. *Environmental Toxicology and Pharmacology*, *5*, 155–172.

Garrett, M. D., & Workman, P. (1999). Discovering novel chemotherapeutic drugs for the third millennium. *European Journal of Cancer*, *35*, 2010–2030.

Greenberg, E. R., Baron, J. A., Karagas, M. R., Stukel, T. A., Nierenberg, D. W., Stevens, M. M., Mandel, J. S., & Haile, R. W. (1996). Mortality associated with low plasma concentration of β-carotene and the effect of oral supplementation. *Journal of the American Medical Association*, *275*, 699–703.

Griffiths, G., Trueman, L., Crowther, T., Thomas, B., & Smith, B. (2002). Onions — A global benefit to health. *Phytotherapy Research*, *16*, 603–615.

Gry, J., Kovatsis, A., Rhodes, M., Rosa, E., Rosner, H., Speijers, G., Søborg, I., & Walker, A. (1998). *Information on inherent food plant toxicants — Guide to resources generated by the EU-AIR-NETTOX project*. Danish Veterinary and Food Administration, Mørkhøj Bygade 19, Søborg, Denmark

Gundgaard, J., Nielsen, J. N., Olsen, J., & Sørensen, J. (2003). Increased intake of fruit and vegetables: estimation of impact in terms of life expectancy and healthcare costs. *Public Health Nutrition*, *6*, 25–30.

Hansen, L., & Boll, P. M. (1986). The polyacetylenic falcarinol as the major allergen in *Schefflera arboricola*. *Phytochemistry*, *25*, 529–530.

Hansen, S. L., Purup, S., & Christensen, L. P. (2003). Bioactivity of falcarinol and the influence of processing and storage on its content in carrots (*Daucus carota* L.). *Journal of the Science of Food and Agriculture*, *83*, 1010–1017.

Hansen-Møller, J., Hansen, S. L., Christensen, L. P., Jespersen, L. Frøkiær, H., Barkholt, V., Haraldsdóttir, J., & Brandt, K. (2002). Quantification of polyacetylenes by LC-MS in human plasma after intake of fresh carrot juice (*Daucus carota* L.). Abstract of presentation at the PSE Symposium on "*Dietary phytochemicals and human health*" Salamanca, Spain, 18–20 April 2002, pp. 58–59

Haraldsdóttir, J., Jespersen, L., Hansen-Møller, J., Hansen, S. L., Christensen, L. P., & Brandt, K. (2002). Recent developments in bioavailability of falcarinol. In: *Health promoting compounds in vegetables and fruit. Proceedings of workshop in Karrebæksminde, Denmark, 6–8 Nov. DIAS report — Horticulture 29,* pp. 24–28. (http://www.agrsci.dk/pvf/Healthpromotingcompounds3/PDF/DJFrap29Have.pdf)

Kassie, F., Uhl, M., Rabot, S., Grasl-Kraupp, B., Verkerk, R., Kundi, M.,

Chabicovsky, M., Schulte-Hermann, R., & Knasmuller, S. (2003). Chemoprevention of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)-induced colonic and hepatic preneoplastic lesions in the F344 rat by cruciferous vegetables administered simultaneously with the carcinogen. *Carcinogenesis*, *24*, 255–261.

Kidmose, U., Hansen, S. L., Nørbæk, R., Christensen, L. P., & Edelenbos, M. (2003). Plant secondary metabolites in organically grown carrots (*Daucus carota* L.) Abstract P2-B16 at conference on New Functional Ingredients and Foods, safety, health and convenience, Copenhagen, Denmark, 9–11 April (http://www.nfif2003.com/posters.htm)

Kitagawa, I., Yoshikawa, M., Hayashi, T., & Taniyama, T. (1983). Chemical studies on crude drug precession. I. On the constituents of ginseng radix rubra. *Journal of the Pharmaceutical Society of Japan*, *103*, 612–622.

Knekt, P., Järvinen, R., Teppo, L., Aromaa, A., & Seppänen, R. (1999). Role of various carotenoids in lung cancer prevention. *Journal of the National Cancer Institute*, *91*, 182–184.

Kobæk-Larsen, M., Fenger, C., Hansen, K., Nissen, I., Diderichsen, A., Thorup, I., van Bieman, M., Vach, W., & Ritskes-Hoitinga, J. (2002). Comparative studies of the histopathology of azoxymethane-induced colonic tumors in three inbred rat strains: BDIX/Orllco, F344/NHsd, and WAG/RijNsd. *Comparative Medicine*, *52*, 50–57.

Krejsa, C. M., Horvath, D., Rogalski, S. L., Penzotti, J. E., Mao, B., Barbosa, F., & Migeon, J. C. (2003). Predicting ADME properties and side effects: the BioPrint approach. *Current Opinion in Drug Discovery & Development*, *6*, 470–480.

Kris-Etherton, P. M., Etherton, T. D., Carlson, J., & Gardner, C. (2002). Recent discoveries in inclusive food-based approaches and dietary patterns for reduction in risk for cardiovascular disease. *Current Opinion in Lipidology*, *13*, 397–407.

Larsen, E., Kharazmi, A., Christensen, L. P., & Brøgger Christensen, S. (2003). An anti-inflammatory galactolipid from rose hip (*Rosa canina* L.) that inhibits chemotaxis of human peripheral blood neutrophils *in vitro*. *Journal of Natural Products*, *66*, 994–995.

Lund, E. (2003). Non-nutritive bioactive constituents of plants: dietary sources and health benefits of glucosinolates. *International Journal for Vitamin and Nutrition Research*, *73*, 135–143.

Matsunaga, H., Katano, M., Yamamoto, H., Fujito, H., Mori, M., & Takata, K. (1990). Cytotoxic activity of polyacetylene compounds in *Panax ginseng* C. A. Meyer. *Chemical & Pharmaceutical Bulletin*, *38*, 3480–3482.

Maynard, M., Gunnell, D., Emmett, P., Frankel, S., & Smith, G. D. (2003). Fruit, vegetables, and antioxidants in childhood and risk of adult cancer: the Boyd Orr cohort. *Journal of Epidemiology and Community Health*, *57*, 218–225.

McLellan, E. A., & Bird, R. P. (1988). Aberrant crypts — potential preneoplastic lesions in the murine colon. *Cancer Research*, *48*, 6187–6192.

Michaud, D. S., Feskanich, D., Rimm, E. B., Colditz, G. A., Speizer, F. E., Willett, W. C., & Giovannucci, E. (2000). Intake of specific carotenoids and risk of lung cancer in 2 prospective US cohorts. *American Journal of Clinical Nutrition*, *72*, 990–997.

Mucci, L. A., Dickman, P. W., Steineck, G., Adami, H. O., & Augustsson, K. (2003). Dietary acrylamide and cancer of the large bowel, kidney, and bladder: absence of an association in a population-based study in Sweden. *British Journal of Cancer*, *88*, 84–89.

Olsson, K., & Svensson, R. (1996). The influence of polyacetylenes on the susceptibility of carrots to storage diseases. *Journal of Phytopathology*, *144*, 441–447.

Omenn, G. S., Goodmann, G. E., Thornquist, M. D., Balmes, J., Cullen, M. R., Glass, A., Keogh, J. P., Meyskens, F. L. Jr., Valanis, B., & Williams, J. H. Jr. (1996). Effects of a combination of β-carotene and vitamin A on lung cancer and cardiovascular disease. *New England Journal of Medicine*, *334*, 1150–1155.

O'Neill, M. E., Carroll, Y., Corridan, B., Olmedilla, B., Granado, F., Blanco, I., Van den Berg, H., Hininger, I., Rousell, A. M., Chopra, M., Southon, S., & Thurnham, D. I. (2001). A European carotenoid database to assess carotenoid intakes and its use in a five-country comparative study. *British Journal of Nutrition*, *85*, 499–507.

Plhak, L. C., & Sporns, P. (1997). Biological activities of potato glycoalkaloids. *Antinutrients and Phytochemicals in Food, ACS Symposium Series*, *662*, 115–126.

Pomerleau, J., McKee, M., Lobstein, T., & Knai, C. (2003). The burden of disease attributable to nutrition in Europe. *Public Health Nutrition*, *6*, 453–461.

Saita, T., Katano, M., Matsunaga, H., Yamamoto, H., Fujito, H., & Mori, M. (1993). The first specific antibody against cytotoxic polyacetylenic alcohol, panaxynol. *Chemical & Pharmaceutical Bulletin*, *41*, 549–552.

Saita, T., Matsunaga, H., Yamamoto, H., Nagumo, F., Fujito, H., Mori, M., & Katano, M. (1994). A highly sensitive enzyme-linked immunosorbent assay (ELISA) for antitumor polyacetylenic alcohol, panaxytriol. *Biological & Pharmaceutical Bulletin*, *17*, 798–802.

Smith, T. K., Lund, E., & Johnson, I.T (1998). Inhibition of dimethylhydrazine-induced aberrant crypt foci and induction of apoptosis in rat colon following oral administration of the glucosinolate sinigrin. *Carcinogenesis*, *19*, 267–273.

Smith, T. K., Mithen, R., & Johnson, I. T. (2003). Effects of Brassica vegetable juice on the induction of apoptosis and aberrant crypt foci in rat colonic mucosal crypts in vivo. *Carcinogenesis*, *24*, 491–495.

St Pierre, M. D., & Bayer, R. J. (1991). The impact of domestication on the genetic variability in the orange carrot, cultivated *Daucus carota* ssp *sativus* and the genetic homogeneity of various cultivars. *Theoretical and Applied Genetics*, *82*, 249–253.

Tavani, A., Bosetti, C., Negri, E., Augustin, L. S., Jenkins, D. J. A., & La Vecchia, C. (2003). Carbohydrates, dietary glycaemic load and glycaemic index, and risk of acute myocardial infarction. *Heart*, *89*, 722–726.

Thomalley, P. J. (2002). Isothiocyanates: mechanism of cancer chemopreventive action. *Anti-Cancer Drugs*, *13*, 331–338.

Trichopoulou, A., Naska, A., Antoniou, A., Friel, S., Trygg, K., & Turrini, A. (2003). Vegetable and fruit: the evidence in their favour and the public health perspective. *International Journal for Vitamin and Nutrition Research*, *73*, 63–69.

Ursin, G., Ziegler, R. G., Subar, A. F., Graubard, B. I., Haile, R. W., & Hoover, R. (1993). Dietary patterns associated with a low-fat diet in the national-health examination follow-up-study — identification of potential confounders for epidemiologic analyses. *American Journal of Epidemiology*, *137*, 916–927.

van't Veer, P., Jansen, M. C. J. F., Klerk, M., & Kok, F. J. (2000). Fruits and vegetables in the prevention of cancer and cardiovascular disease. *Public Health Nutrition*, *3*, 103–107.

Williams, D. E. M., Wareham, N. J., Cox, B. D., Byrne, C. D., Hales, C. N., & Day, N. E. (1999). Frequent salad vegetable consumption is associated with a reduction in the risk of diabetes mellitus. *Journal of Clinical Epidemiology*, *52*, 329–335.

Wong, H. R., & Menendez, I. Y. (1999). Sesquiterpene lactones inhibit inducible nitric oxide synthase gene expression in cultured rat aortic smooth muscle cells. *Biochemistry Biophysics Research Communications*, *262*, 375–380.

Wright, M. E., Mayne, S. T., Swanson, C. A., Sinha, R., & Alavanja, M. C. R. (2003). Dietary carotenoids, vegetables, and lung cancer risk in women: the Missouri Women's Health Study (United States). *Cancer Causes and Control*, *14*, 85–96.

Ziegler, R. G., Colavito, E. A., Hartge, P., McAdams, M. J., Schoenberg, J. B., Mason, T. J., & Fraumeni, J. F. Jr. (1996). Importance of alpha-carotene, beta-carotene, and other phytochemicals in the etiology of lung cancer. *Journal of the National Cancer Institute*, *88*, 612–615.