JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Inhibitory Effects of Feeding with Carrots or (–)-Falcarinol on Development of Azoxymethane-Induced Preneoplastic Lesions in the Rat Colon

MORTEN KOBÆK-LARSEN,*,† LARS P. CHRISTENSEN,‡ WERNER VACH,§
JELMERA RITSKES-HOITINGA,† AND KIRSTEN BRANDT||

Biomedical Laboratory, University of Southern Denmark, Winsloewparken 23, DK-5000 Odense C, Denmark, Department of Food Science, Danish Institute of Agricultural Sciences, Research Centre Aarslev, Kirstinebjergvej 10, DK-5792 Aarslev, Denmark, Department of Statistics, University of Southern Denmark, Sdr. Boulevard 23A, DK-5000 Odense C, Denmark, and School of Agriculture, Food and Rural Development, University of Newcastle upon Tyne, Agriculture Building, Newcastle upon Tyne NE1 7RU, United Kingdom

The effects of intake of dietary amounts of carrot or corresponding amounts of (−)-(3*R*)-falcarinol from carrots on development of azoxymethane (AOM)-induced colon preneoplastic lesions were examined in male BDIX rats. Three groups of eight AOM-treated rats were fed the standard rat feed Altromin supplemented with either 10% (w/w) freeze-dried carrots with a natural content of 35 μg falcarinol/g, 10% maize starch to which was added 35 μg falcarinol/g purified from carrots, or 10% maize starch (control). After 18 weeks, the animals were euthanized and the colon was examined for tumors and aberrant crypt foci (ACF), which were classified into four size classes. Although the number of small ACF was unaffected by the feeding treatments, the numbers of lesions as a function of increasing size class decreased significantly in the rats that received one of the two experimental treatments, as compared with the control treatment. This indicates that the dietary treatments with carrot and falcarinol delayed or retarded the development of large ACF and tumors. The present study provides a new perspective on the known epidemiological associations between high intake of carrots and reduced incidence of cancers.

KEYWORDS: ***Daucus carota*; aberrant crypt foci; (3*R*)-falcarinol; BDIX rats; natural toxicant; colon carcinogenesis**

INTRODUCTION

While it is well-known from epidemiological studies that a high intake of vegetables and fruits reduces the risk of cancer (*1*−*4*), knowledge is still very limited about which components in these foods are primarily responsible for this reduction. Highly bioactive plant secondary metabolites are mostly known for their toxicities at high concentrations (*5*). However, one of the many theories in this field predicts that these compounds could have cancer-preventing effects at lower concentrations, corresponding to the levels found naturally in food (*6*), as it has already been shown for glucosinolates from *Brassica* vegetables (*7*).

Several epidemiological studies imply specifically the intake of carrots as important for this preventive effect. Many studies have been based on the hypothesis that the antioxidant β-carotene was responsible for this. However, subsequent intervention studies ruled out this explanation, since supplementation with β-carotene does not reduce cancer incidence and, in some cases, even increases the risk for this disease (*8*−*10*). Even though carrots are the major source of β-carotene in the diet in Northern Europe and North America, they also contain a group of bioactive polyacetylenes, of which falcarinol (**Figure 1**) clearly is the most bioactive of the carrot polyacetylenes (*11*−*14*).

In the human diet, carrots are the almost exclusive dietary source of falcarinol. A recent in vitro study aiming to screen for potentially health promoting compounds from vegetables showed that falcarinol could stimulate differentiation of primary mammalian cells in concentrations between 1 and 50 ng/mL, while toxic effects were found above 100 ng/mL (*11*). This biphasic effect (hormesis) of falcarinol on cell proliferation is fully in accordance with the hypothesis that most highly bioactive compounds exhibit hormesis (*15*). Ingestion of carrot juice containing 13 μg falcarinol/mL by human volunteers resulted in a plasma concentration of falcarinol of 2 ng/mL for several hours (*16*). This is within the range where the in vitro data indicate that a potentially beneficial physiological effect would be expected (*11*). Earlier studies showed that physiologically

* To whom correspondence should be addressed. Tel: +45 65 50 37 21. Fax: +45 65 90 68 21. E-mail: mkobaek@health.sdu.dk.
† Biomedical Laboratory, University of Southern Denmark.
‡ Danish Institute of Agricultural Sciences.
§ Department of Statistics, University of Southern Denmark.
|| University of Newcastle upon Tyne.

10.1021/jf048519s CCC: $30.25 © 2005 American Chemical Society
Published on Web 02/05/2005

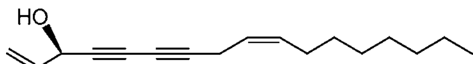

Figure 1. Chemical structure of (3*R*)-(9*Z*)-heptadeca-1,9-diene-4,6-diyne-3-ol [(−)-falcarinol] isolated from carrots and tested in the present study.

relevant concentrations of falcarinol had a pronounced cytotoxic effect on several human tumor cell lines in vitro (*12*−*14*). Carrots and carrot extracts can also reduce the incidence of hepatomas (*17*), although only minimal curative effects of falcarinol were found when a highly invasive form of brain cancer in mice was studied (*12*). A more detailed account of the background for the present study has recently been described (*6*).

In the present study, we examined the possible cancer preventive effects of realistic dietary amounts of carrot or corresponding amounts of falcarinol from carrots on the development of azoxymethane (AOM)-induced aberrant crypt foci (ACF) and tumors in male BDIX rats. ACF are precancerous lesions often used as biomarkers for colon carcinogenesis (*18*), and we used the distribution among different size classes as a measure of the extent of their progress toward actual tumors.

MATERIALS AND METHODS

Animals. Male rats from the BDIX/OrlIco strain with a certified health report were purchased from IFFA CREDO (L'Abresle, France). The animals were 8 weeks old at the time of the first injection with AOM, as described in earlier studies (*19*). All animals were housed in groups of two rats in Macrolon type III cages (Scanbur A/S, Køge, Denmark). AOM-treated animals were housed inside an isolator [Isotec type 13366 (M50), Harlan, The Netherlands] with negative pressure (3 mm H_2O) in order to protect the personnel and the environment from this carcinogen and its metabolites. Two weeks after the final AOM injection, the animals were moved outside the isolator and housed in the same animal room. Rats that did not receive AOM injections were housed outside the isolator in the same animal room throughout the study. The animals were kept under standard laboratory conditions: room temperature, 20−24 °C; relative humidity, 50−60%; and 12 h light/dark cycle (lights on from 6.00 to 18.00 h). The temperature and relative humidity inside the isolator were not recorded separately. The bedding consisted of irradiated aspen wood chips (Tapvei, Oy, Kaavi, Suomi), and the cages were changed twice a week both inside and outside the isolator. While outside the isolator, the rats were given environmental enrichment as aspen wood shavings and/or wooden blocks (Tapvei) twice a week. Animals in the isolator were allowed free access to acidified tap water (acidified with HCl to pH 3 in order to reduce bacterial growth) via water bottles, which were changed once during their 8 week stay in the isolator (full details given in ref *19*). Outside the isolator, the rats had free access to nonacidified water in water bottles, which were changed once a week. Food was available ad libitum, both inside and outside the isolator. Fresh food was given at least once a week on top of the remaining food.

Plant Material. Carrots (cv. Bolero) were grown organically at Research Centre Aarslev in 2002. Tops and bottoms were removed from fresh, washed carrots, which were then shredded, freeze-dried below 50 °C (Danish Freeze-Dry, Kirke Hyllinge, Denmark), and packed in sealed aluminum foil pouches until use.

Extraction, Isolation, and Quantification of (3*R*)-(9*Z*)-Heptadeca-1,9-diene-4,6-diyne-3-ol [(−)-Falcarinol]. Falcarinol was isolated from carrots according to the procedure described by Hansen et al. (*11*) with a few modifications. Eight kilograms of freeze-dried carrots were extracted twice with 12 L of ethyl acetate (99.9% HPLC grade, Aldrich-Chemie, Steinheim, Germany) for 24 h at 8 °C. The combined extracts were filtered and concentrated in vacuo (35 °C) under dim light. The extract (26 g) was chromatographed on silica gel, eluting with *n*-hexane, *n*-hexane−ethyl acetate (v/v) (9:1, 4:1, 7:3, 3:2, 1:1, 2:3, 3:7, 1:4), ethyl acetate, and finally with CH_3OH [99.9% high-performance liquid chromatography (HPLC) grade, Aldrich-Chemie]. Fractions containing crude falcarinol were further purified by preparative reversed phase (RP) HPLC on a Develosil ODS-HG-5 (RP-18, 250 mm × 20 mm i.d., Nomura Chemical Co., Seto, Japan) column using the following stepwise gradient: CH_3OH-H_2O (v/v), 40:60, 50:50, 60:40, 70:30, 80:20, and 100:0, yielding 235 mg of (−)-falcarinol (purity of >99%, as determined by analytical RP-HPLC). Monitoring was performed at 256 nm; flow rate, 6 mL/min. (−)-Falcarinol was obtained as a colorless oil and identified by optical rotation, UV, mass spectrometry (MS) [gas chromatography (GC)−MS (EI, 70 eV)], one-dimensional and two-dimensional NMR (^1H and ^{13}C NMR and $^1H-^1H$ and $^1H-^{13}C$ correlation spectroscopy), and the complete spectral data set corresponded fully with literature values (*20*−*25*). The optical rotation for the isolated falcarinol was found to be levorotatory ($[\alpha]_D^{20}$ −36.8°, *c* 0.88, $CHCl_3$), which is consistent with literature values {$[\alpha]_D$ −36.6°, *c* 0.92, $CHCl_3$ (*24*); $[\alpha]_D$ −36.93°, *c* 0.77, $CHCl_3$ (*25*)} for the 3*R* configuration of falcarinol.

Falcarinol was quantified in carrot samples by analytical RP-HPLC. Extraction of carrot samples for analytical RP-HPLC analysis was performed by extracting 10 g of freeze-dried carrots twice with 100 mL of ethyl acetate according to the procedure of Hansen et al. (*11*). Analytical RP-HPLC was performed on a Merck D-7000 Hitachi HPLC system using diode array detection. Separations were performed on a LiChrospher 100 RP-18 column (particle size 5 μm; 244 mm × 4 mm i.d.; Merck, Darmstadt, Germany) at 35 °C, by gradient elution with solvent A [CH_3OH-H_2O−trifluoroacetic acid (TFA; Sigma Chemical Co., St. Louis, MO) (v/v/v), 95:4.5:0.5], solvent B [H_2O-CH_3OH−TFA (v/v/v), 94.5:5.0:0.5], and solvent C [CH_3OH−tetrahydrofuran (99.9% HPLC grade, Sigma Chemical Co.) (v/v), 50:50]. Elution profile: 0 min 26% B and 19% C, 20 min 11% B and 19% C, 21−26 min 0% B and 0% C, 29 min 40% B and 0% C and 39 min 40% B and 0% C. All changes in the programmed gradient were linear; flow, 1.0 mL/min. The HPLC samples were cooled to 10 °C in the autosampler, and 20 μL of sample was injected. Mobile phases were degassed with ultrasound for 20 min before use. Falcarinol eluted at 26 min and had the following UV-maxima: λ_{max} 229, 243, 256 nm. Falcarinol was identified by peak addition of an authentic standard and quantified in carrot extract samples using falcarinol as external standard. The validity of the HPLC method was checked with regard to accuracy, linearity, and precision.

Rat Diet. Before the main experiment, four male BDIX rats were given free access to both a standard rat feed (Altromin) and freeze-dried carrots. The consumption of each type of feed was recorded daily, and after a run-in period of 14 days, the voluntary intake of freeze-dried carrots stabilized around 10−20% of the daily total feed intake.

For the main experiment, the standard rat feed (Altromin 1234, Chr. Petersen Inc., Denmark) was pulverized before addition of the supplementary materials, to ensure that the rats did not select one component at the expense of another. Diet group 1 contained standard rat feed supplemented with 10% pulverized freeze-dried carrots containing 35 μg falcarinol/g; diet group 2 contained standard rat feed supplemented with 10% maize starch and purified falcarinol corresponding to 10% carrots; and diet group 3 (control group) contained standard rat feed supplemented with 10% maize starch. Because the isolated falcarinol for diet group 2 was applied to the diet in the form of an ethanol solution, the diets for the two other groups were also treated with the same amount of ethanol. Each time, portions of 20 kg diet were prepared for each of the three groups and 2 L of 96% ethanol or ethanol solution was applied to the diets using an atomizer, after which the portion was allowed to dry at room temperature for approximately 1 h in darkness before being packed in sealed aluminum foil pouches. The Altromin and freeze-dried carrots were stored at −20 °C before preparations. The prepared diets were stored at room temperature, mixed well before use, and used for approximately 1 month before new diets were prepared. The content of falcarinol in the rat diets (groups 1 and 2) was measured by analytical HPLC before use and at approximately monthly intervals throughout the experiment. No significant differences in the content of falcarinol in either diet were observed during the experiment.

Design of the Rat Feeding Experiments. A total of 30 rats were divided into three groups that received different diets, starting 10 days before the first AOM injection. AOM purchased from Sigma Chemical Co. was diluted with sterile 0.9% NaCl to a concentration of 5 mg/mL at the Central Pharmacy of the Odense University Hospital (Odense,

Denmark). The AOM solution was stored for about 1 h at room temperature before being injected. Eight of the 10 animals in each treatment group were given weekly subcutaneous (s.c.) injections of freshly prepared AOM at a dose of 15 mg/kg body weight for a period of 2 × 2 weeks separated by a 1 week break (*19*). The range of the injection volume used was 0.4 mL at the start and 1.0 mL at the end of the AOM treatments. Two rats in each treatment group were injected with a volume of sterile 0.9% NaCl related to the body weight.

Autopsy Procedures. The rats were euthanized after 18 weeks, when the first symptoms of cancer (blood in stools) were observed in a few animals, and autopsied to examine for macroscopic alterations. The animals were killed in 100% CO_2, after they had been anaesthetized (duration max, 30 min) s.c. with a mixture of 0.3 mL of Hypnorm/kg rat (0.095 mg/kg fentanyl citrate and 3 mg/kg fluanisone, JANSSEN Animal Health, Beerse, Belgium) and 0.675 mL Dormicum/kg rat (3.375 mg/kg midazolam, Dumex-Alpharma, Oslo, Norway). Immediately after death, selected organs were fixed in 4% phosphate-buffered formaldehyde, pH 7.4, for later histopathological examination. The total length of the intestine was measured, and it was then cut longitudinally, rinsed in 0.9% NaCl solution, cut into two equally sized pieces, and pinned on a cork slab. Before fixation, the large intestine was evaluated for macroscopic neoplasms, where diameter and location in the intestine were registered.

Identification and Quantification of Tumors and ACF. After fixation of the large intestine, Giemsa stain [6 mL of stock solution (The Central Pharmacy at the Odense University Hospital) in 50 mL of phosphate-buffered saline (PBS), pH 7.2, for 15 min] was used to visualize the ACF, and excess stain was rinsed off with PBS. The tissue was placed with the luminal side up in a Petri dish with enough PBS to cover the tissue. The total numbers of ACF and tumors for each section of the large intestinal were counted independently by two persons, blinded to treatment modality, by using a stereomicroscope at 40× magnification. The aberrant crypts were distinguished by their increased size and thicker and deeply stained epithelial lining as compared with normal crypts. An ACF may consist of one to several crypts, and in the present study, the ACF were classified as small (1−3 crypts), medium (4−6 crypts), or large (more than seven crypts), while neoplasms larger than 1 mm in diameter were classified as tumors. Within each class of lesions, the variation coefficient of the single counts between the two persons was less than 10%. The counts of the two persons were averaged.

The tumors were fixed in 4% (v/v) formaldehyde buffered with 0.075 M sodium phosphate (pH 7) and embedded in paraffin. The tissues were cut into 5 μm sections and were stained with haematoxylin and eosin. Additional sections were cut until characterization of the neoplasm was certain.

Statistical Analysis. The counts of the four different size classes of (pre)neoplastic lesions were normalized in the carrot and falcarinol treatment groups by dividing with the class specific mean count in the control group. The four size classes were scored from one to four with increasing lesion size. The effect of the class on the normalized counts in the two treatment groups (carrot and falcarinol) was assessed by a regression model using the size class score and the treatment group as covariates. The correlation among the four size classes of ACF/tumors within each individual rat has been taken into account in this analysis by using robust variance estimates.

RESULTS

Although both (+)- and (−)-falcarinol have been isolated from different plants and appear to occur regularly in plants of the Araliaceae (*12*, *14*, *25*) and Apiaceae (*11*, *21*, *23*, *26*), the optical rotation of falcarinol, and hence the absolute configuration, when isolated from plants has often not been determined. To the best of our knowledge, the absolute configuration of falcarinol in carrots has not been determined. Falcarinol was isolated from carrots by preparative HPLC and identified by spectroscopic means to be levorotatory falcarinol (see Materials and Methods). Thus, (−)-falcarinol (**Figure 1**) tested in the present study on rats on development of AOM-induced colon preneoplastic lesions possesses the 3*R* configuration (*24*, *25*).

Table 1. Numbers of (Pre)Neoplastic Lesions of Different Sizes in Colons of Rats Fed with Different Supplements; 10% Added to Their Respective Diets, Results Expressed as Means ± Standard Deviations

treatment (supplement used, 10% mixed in Altromin diet)	small[a] (1–3 crypts)	medium[a] (4–6 crypts)	large[a] (>7 crypts)	tumors[a] (≥1 mm diameter)
freeze-dried carrots containing 35 μg falcarinol/g	83 ± 28	48 ± 19	13 ± 8	0.9 ± 1.0
maize starch where 35 μg falcarinol/g was added	101 ± 43	59 ± 35	18 ± 12	0.8 ± 0.7
maize starch (control)	98 ± 17	67 ± 16	21 ± 9	1.4 ± 1.1

[a] None of the differences between the three diets were significant when analyzed within one size class or on summations of total numbers across size classes.

Animal weight gain was identical in all treatments (data not shown), and no mortality, signs of distress, or disease nor gross abnormalities were observed. The histopathological aspects of the model using the same experimental design as the control treatment have been reported previously (*19*). Neither tumors nor ACF were observed in the two animals per dietary treatment that were injected with 0.9% NaCl solution (data not shown).

The numbers of each size class of ACF and tumors found are presented in **Table 1**. In every dietary treatment, while the numbers of individual lesions decreased very much with increasing size of the lesion, each size class still represented a comparable amount of tissue with aberrant growth pattern. For example, a tumor typically contained the same amount of tissue as 50−200 small ACF.

All tumors identified macroscopically were subsequently identified by histological examination as either adenomas or carcinomas, with an approximately equal number of each type in each treatment group (data not shown). All of the carcinomas were restricted to invading the submucosa.

The number of animals was too small to determine whether the treatments resulted in differences in the numbers of each of the various lesion types found or in the sums of all types (**Table 1**). However, the carrot and falcarinol treatments showed a significant ($P = 0.028$) tendency to reduced numbers of (pre)-cancerous lesions with an increasing size of lesions (**Figure 2**), from no difference relative to control for the smallest ACF, to a one-third reduction for the fully developed tumors.

DISCUSSION

The relation between lesion size and treatment effect corresponds to the trend expected if the bioactive compound is capable of reducing the growth of precancerous lesions, under conditions where the preventive effect is not sufficient to prevent the initial proliferation (which takes place during the actual period of challenge with the carcinogen). The observed results could either be due to a decreased growth rate or survival of the individual tumor precursor cells (cytotoxicity), to a decrease in the rate of progression through the stages of carcinogenesis, or to enhancement of one or more of the rats' relevant immune system components. Earlier studies of falcarinol, primarily in vitro studies of its potential use as a drug, have already shown effects corresponding to each of these mechanisms (*5*, *27*), although their relative relevance in vivo is still unknown.

Our results thus indicate that consumption of carrot led to a reduced risk of colorectal cancer in this rat model, which is in line with the epidemiological data indicating that carrot consumption provides a protective effect on the development of cancers. They also show that the effect of falcarinol alone was of the same magnitude as that of the entire carrots (**Figure**

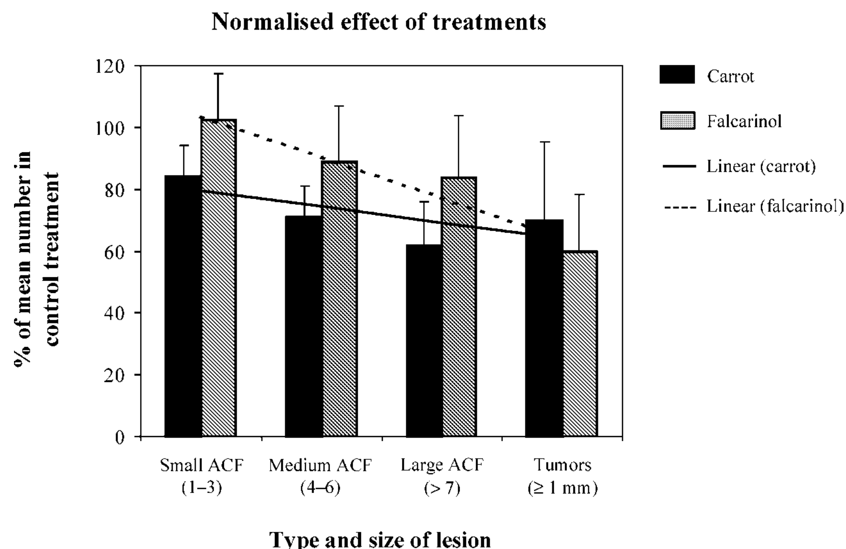

Figure 2. Effect of treatments with carrot or falcarinol on the average numbers per animal of four types of (pre)cancerous lesions in rat colons, each size class representing increasingly advanced steps on the progression toward cancer. The size of ACF was measured as the number of crypts found on a corresponding area of normal colon tissue. The smallest tumors correspond to an ACF size of approximately 20. The trend for reduced relative numbers with increasing size of lesion was significant at $P = 0.028$.

2), which supports the hypothesis that falcarinol is an active protective substance in carrots. Falcarindiol, which is the quantitatively predominant polyacetylene in carrots (*20*), has also been shown to possess cytotoxic (*12*, *28*) and antimutagenic activity in vitro (*29*), although it appears to be much less bioactive than falcarinol (*11*−*14*). The bioactivity of falcarindiol-3-acetate a further polyacetylene in carrots (*20*) has so far not been investigated. The possible mode of action of falcarinol may be related to its hydrophobicity and its ability to form an extremely stable carbocation with the loss of water, thereby acting as a very reactive alkylating agent toward proteins and other biomolecules (*30*). This may also explain the highly allergenic properties of falcarinol (*30*). A similar mode of action may be possible for falcarindiol and falcarindiol-3-acetate, although the possibility to generate two active centers for nucleophilic attack reduces the lipophilic character of these compounds and hence their reactivity, in accordance with the observed nonallergenic properties of falcarindiol (*30*). So the physiological effects of falcarindiol and falcarindiol-3-acetate are expected to be qualitatively similar but quantitatively less than those found for falcarinol, and furthermore, they may even interact with falcarinol in an antagonistic manner thereby affecting its effectiveness. This could explain the possible, although not significant, differences in the effect and the trend observed between the treatments with falcarinol and the carrot diet (**Figure 1**). Other relevant bioactive compounds in carrots that have been considered in this context, but about which even less information is available (*6*), are isocoumarins such as 6-methoxymellein and a large number of mono- and sesquiterpenes.

As far as we know, these are the first results directly indicating falcarinol as the primary cancer protective substance from carrots. As a natural pesticide, falcarinol is best known for its toxic properties, which are observed at high concentrations, and falcarinol is listed as a toxicant in the Nettox database (*5*). It is possible that falcarinol could be beneficial and act as a cancer preventive substance in the relatively low amounts found in food, even though it is harmful at high intake levels, just as it has been shown for other highly bioactive food components, such as ethanol (*4*).

To confirm the tendencies presented here in future studies, higher numbers of animals and/or other models are needed. A power calculation based on the reported data shows that to obtain a 90% power for each of the medium and large ACFs, 25 animals per treatment group would be needed. For tumors, the corresponding number would be 3−4 times higher, due to the small number of tumors per animal.

The amount of carrot in the diet was chosen to correspond to the voluntary intake when rats were given free choice between freeze-dried carrots and Altromin. This ensured that the results would be physiologically relevant, an issue that is particularly important when studying food components. Imposing a nutritionally deficient diet could by itself inhibit the development of cancer (*31*), but because rats are known to be able to efficiently detect and avoid nutritionally deficient diets (*32*), the described procedure ensured that the carrot treatment was nutritionally adequate. Still, if the use of starch as a substitute for carrot in the feed for the other treatments caused so large a difference in the nutritional value between treatments that the formation of (pre)neoplastic lesions was affected, it would have inhibited carcinogenesis in the starch-fed rats. This implies that in our study we might be underestimating the difference between the positive control (carrot) treatment and the negative control (starch) treatment, so we have to regard the observed protective effect as a minimum estimate. Ten percent of the dry weight of the daily food intake corresponds to a daily human consumption of 400−600 g fresh weight of carrot. This is the intake level of fruit and vegetables recommended by many official agencies responsible for food safety and quality, to decrease the risk of cancer and other diseases (*3*).

The presence of equally high numbers of small ACF in all treatments could be due to a saturation effect: that the intensive AOM treatment might have induced the maximal number of preneoplasms that are able to develop in this system, irrespective of possible differences in susceptibility related to the feed treatments. If this has been the case, reduction of the amounts of AOM used in similar future studies may facilitate quantification of effects of treatments on the total number of ACF formed.

If future studies confirm the present results, both in terms of protection against cancer and absence of indications of toxicity, it would indicate that the moderately increased falcarinol content in food could reduce the risk of cancer. The present experiment together with the experiments leading to it (*6*) thus highlights

the need to include the many overlooked bioactive food compounds such as falcarinol in ongoing and planned research on improvement of food safety and quality, including basic studies on the functioning of human cells during normal and abnormal development.

LITERATURE CITED

(1) Trichopoulou, A.; Naska, A.; Antoniou, A.; Friel, S.; Trygg, K.; Turrini, A. Vegetable and fruit: The evidence in their favour and the public health perspective. *Int. J. Vitam. Nutr. Res.* **2003**, *73*, 63–69.

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

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

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

(5) Gry, J.; Kovatsis, A.; Rhodes, M.; Rosa, E.; Rosner, H.; Speijers, G.; Søborg, I.; Walker, A. *Information on Inherent Food Plant Toxicants—Guide to Resources Generated by the EU-AIR-NETTOX Project*; Danish Veterinary and Food Administration: Søborg, Denmark, 1998.

(6) Brandt, K.; Christensen, L. P.; Hansen-Møller, J.; Hansen, S. L.; Haraldsdóttir, J.; Jespersen, L.; Purup, S.; Kharazmi, A.; Barkholt, V.; Frøkiær, H.; Kobæk-Larsen, M. Health promoting compounds in vegetables and fruits. *Trends Food Sci. Technol.* **2004**, *15*, 384–393.

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

(8) 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. Effects of a combination of β-carotene and vitamin A on lung cancer and cardiovascular disease. *New Engl. J. Med.* **1996**, *334*, 1150–1155.

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

(10) The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group. The effect of vitamin E and β-carotene on the incidence of lung cancer and other cancers in male smokers. *New Engl. J. Med.* **1994**, *330*, 1029–1035.

(11) Hansen, S. L.; Purup, S.; Christensen, L. P. Bioactivity of falcarinol and the influence of processing and storage on its content in carrots (*Daucus carota* L.). *J. Sci. Food Agric.* **2003**, *83*, 1010–1017.

(12) Bernart, M. W.; Cardellina, J. H., II; Balaschak, M. S.; Alexander, M.; Shoemaker, R. H.; Boyd, M. R. Cytotoxic falcarinol oxylipins from *Dendropanax arboreus*. *J. Nat. Prod.* **1996**, *59*, 748–753.

(13) Saita, T.; Katano, M.; Matsunaga, H.; Yamamoto, H.; Fujito, H.; Mori, M. The first specific antibody against cytotoxic polyacetylenic alcohol, panaxynol. *Chem. Pharm. Bull.* **1993**, *41*, 549–552.

(14) Matsunaga, H.; Katano, M.; Yamamoto, H.; Fujito, H.; Mori, M.; Takata, K. Cytotoxic activity of polyacetylene compounds in *Panax ginseng* C. A. Meyer. *Chem. Pharm. Bull.* **1990**, *38*, 3480–3482.

(15) Calabrese, E. J.; Baldwin, L. A. Applications of hormesis in toxicology, risk assessment and chemotherapeutics. *Trends Pharmacol. Sci.* **2002**, *23*, 331–337.

(16) Haraldsdóttir, J.; Jespersen, L.; Hansen-Møller, J.; Hansen, S. L.; Christensen, L. P.; Brandt, K. Recent developments in bioavailability of falcarinol. In *Health Promoting Compounds in Vegetables and Fruit*; Brandt, K., Åkesson, B., Eds; Proceedings of the workshop in Karrebæksminde, Denmark, 6–8 Nov; DIAS Report—Horticulture 29, 24–28 (http://www.agrsci.dk/pvf/Healthpromotingcompounds3/PDF/DJFrap29Have.pdf).

(17) Rieder, A.; Adamek, M.; Wrba, H. Delay of diethylnitrosamine-induced hepatoma in rats by carrot feeding. *Oncology* **1983**, *40*, 120–123.

(18) Corpet, D. E.; Tache, S. Most effective colon cancer chemopreventive agents in rats: A systematic review of aberrant crypt foci and tumor data, ranked by potency. *Nutr. Cancer Int. J.* **2002**, *43*, 1–21.

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

(20) Czepa, A.; Hofmann, T. Structural and sensory characterization of compounds contributing to the bitter off-taste of carrots (*Daucus carota* L.) and carrot puree. *J. Agric. Food Chem.* **2003**, *51*, 3865–3873.

(21) Nakano, Y.; Matsunaga, H.; Saita, T.; Mori, M.; Katano, M.; Okabe, H. Antiproliferative constituents in Umbelliferae plants II. Screening for polyacetylenes in some Umbelliferae plants, and isolation of panaxynol and falcarindiol from the root of *Heracleum moellendorffii*. *Biol. Pharm. Bull.* **1998**, *21*, 257–261.

(22) Hirakura, K.; Morita, M.; Nakajima, K.; Ikeya, Y.; Mitsuhashi, H. Three acetylenic compounds from roots of *Panax ginseng*. *Phytochemistry* **1992**, *31*, 899–903.

(23) Nitz, S.; Spraul, M. H.; Drawert, F. C_{17} Polyacetylenic alcohols as the major constituents in roots of *Petroselinum crispum* Mill. ssp. *tuberosum*. *J. Agric. Food Chem.* **1990**, *38*, 1445–1447.

(24) Zheng, G.; Lu, W.; Aisa, H. A.; Cai, J. Absolute configuration of falcarinol, a potent antitumor agent commonly occurring in plants. *Tetrahedron Lett.* **1999**, *40*, 2181–2182.

(25) Terada, A.; Tanoue, Y.; Kishimoto, D. (−)-(9*Z*)-1,9-Heptadecadiene-4,6-diyn-3-ol as a principal component of the resinous sap of *Evodiopanax innovans* Nakai, Japanese name, Takanotsume, and its role in an ancient golden varnish of Japan. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 2977–2980.

(26) Bohlmann, F.; Burkhardt, T.; Zdero, C. *Naturally Occurring Acetylenes*; Academic Press: London, 1973; pp 464–485.

(27) Kuo, Y.-C.; Lin, Y. L.; Huang, C.-P.; Shu, J.-W.; Tsai, W.-J. A tumor cell growth inhibitor from *Saposhnikovae divaricata*. *Cancer Invest.* **2002**, *20*, 955–964.

(28) Cunsolo, F.; Ruberto, G. Bioactive metabolites from Sicilian marine fennel, *Crithmum maritimum*. *J. Nat. Prod.* **1993**, *56*, 1598–1600.

(29) Miyazawa, M.; Shimamura, H.; Bhuva, R. C.; Nakamura, S.; Kameoka, H. Antimutagenic activity of falcarindiol from *Peucedanum praeruptorum*. *J. Agric. Food Chem.* **1996**, *44*, 3444–3448.

(30) Hansen, L.; Hammershøy, O.; Boll, P. M. Allergic contact dermatitis from falcarinol isolated from *Schefflera arboricola*. *Contact Dermatitis* **1986**, *14*, 91–93.

(31) DeWille, J. W.; Waddell, K.; Steinmeyer, C.; Farmer, S. J. Dietary fat promotes mammary tumorigenesis in MMTV/v-Ha-ras transgenic mice. *Cancer Lett.* **1993**, *69*, 59–66.

(32) Wang, Y.; Cummings, S. L.; Gietzen, D. W. Temporal-spatial pattern of c-Fos expression in the rat brain in response to indispensable amino acid deficiency II. The learned taste aversion. *Mol. Brain Res.* **1996**, *40*, 35–41.

Received for review September 6, 2004. Revised manuscript received December 20, 2004. Accepted December 22, 2004. The support of the Danish Research Council for Agricultural and Veterinary Research, Danish Food Technology Initiative (FØTEK 3) Grant 2011-00-0040, and of the Development Centre Aarslev are gratefully acknowledged.

JF048519S