Walnuts Have Potential for Cancer Prevention and Treatment in Mice\textsuperscript{1−3}

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Abstract

Cancer may not be completely the result of novel or inherited genetic mutations but may in fact be a largely preventable disease. Researchers have identified bioactive compounds, including n−3 ω-3 fatty acids, tocopherols, β-sitosterol, and pedunculagin, that are found in walnuts and that have cancer-prevention properties. Mouse studies in which walnuts were added to the diet have shown the following compared with the control diet: 1) the walnut-containing diet inhibited the growth rate of human breast cancers implanted in nude mice by ~80%; 2) the walnut-containing diet reduced the number of mammary gland tumors by ~60% in a transgenic mouse model; 3) the reduction in mammary gland tumors was greater with whole walnuts than with a diet containing the same amount of n−3 fatty acids, supporting the idea that multiple components in walnuts additively or synergistically contribute to cancer suppression; and 4) walnuts slowed the growth of prostate, colon, and renal cancers by antiproliferative and antiangiogenic mechanisms. Cell studies have aided in the identification of the active components in walnuts and of their mechanisms of action. This review summarizes these studies and presents the notion that walnuts may be included as a cancer-preventive choice in a healthy diet. J. Nutr. doi: 10.3945/jn.113.188466.

\textsuperscript{1} Presented at "What Comes First: The Food or the Nutrient," as a satellite session to the American Society for Nutrition Scientific Sessions and Annual Meeting at Experimental Biology held in Boston, MA, on 19 April 2013. The satellite session and supplement publication were supported by the California Walnut Commission (CWC). All session speakers received travel funding and/or honoraria for participation in the meeting and manuscript preparation. The views expressed are those of the authors. Because the symposium was held on the day of the city lockdown in the search for the Boston Marathon bomber, presentation was to a limited audience; Dr. Katz was not present. The recorded presentations are available at http://www.nutrition.org/education-and-professional-development/archived-content-from-past-meetings-and-professional-development-events/asn-at-eb-2013/recorded-sessions/satellite-session-what-comes-first-the-food-or-the-nutrient/. The CWC and all of the presenters express their solidarity with the people of Boston, and particularly with those killed or injured at the Boston Marathon. The Supplement Coordinator for this supplement was David R. Jacobs, Jr. Supplement Coordinator disclosures: David Jacobs, PhD, is a consultant at the California Walnut Commission (member of the Scientific Advisory Council). Dr. Jacobs' travel expense to Experimental Biology 2013 was paid by the California Walnut Commission. Dr. Jacobs is otherwise employed by the University of Minnesota and is supported by government grants. This supplement is the responsibility of the Guest Editor to whom the Editor of The Journal of Nutrition has delegated supervision of both technical conformity to the published regulations of The Journal of Nutrition and general oversight of the scientific merit of each article. The Guest Editor for this supplement was Kevin Schalinske. Guest Editor disclosure: Kevin Schalinske had no conflicts to disclose. Publication costs for this supplement were defrayed in part by the payment of page charges. This publication must therefore be hereby marked “advertisement” in accordance with 18 USC section 1734 solely to indicate this fact. The opinions expressed in this publication are those of the authors and are not attributable to the sponsors or the publisher, Editor, or Editorial Board of The Journal of Nutrition.

\textsuperscript{2} This work has been supported by grants from the American Institute for Cancer Research, the California Walnut Commission, Department of Defense Breast Cancer Research Program grants DAMD17-03-1-0681 and W81XWH-10-1-0697, and by NIH-NCI R01CA114018, NIH-NCRR 5P20RR020180, and 5P20RR016477. The sponsors had no influence on study designs, interpretation, or publication of results. The opinions expressed in this report are those of the author and do not necessarily represent the positions of any sponsor.

\textsuperscript{3} Author disclosures: W. E. Hardman, no conflicts of interest.

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within a generation after migration to a different area, but diet, nutrition, and physical activity patterns do change (3, p. 25). Statistical analyses showed that dietary changes had the strongest correlation with the changing cancer incidence.

An urban economy is different from a rural economy in ways that could be associated with increased risks for cancer. The quality of the food in an urban economy is different from that found in a rural economy. People in rural areas produce and consume much of the food in a region, whereas people in urban areas generally produce little of the food and consume more processed and less "native" food. The urban diet typically contains higher quantities of energy-dense food (more fats, oils, and sugars) and less whole grain and starchy foods than found in a rural diet. Finally, living in an urban area often includes an increasingly sedentary lifestyle. These factors combine to contribute to the accumulation of excess body weight and to increased carcinogenesis as well as to other chronic diseases (3, p. 6–10).

Consumption of various foods as part of the usual diet has been recommended to help fight cancer (4,5). These beneficial foods include fruits and vegetables, whole grains, and nuts. However, scientists want to find "the" component of whole foods that is responsible for the reduction in cancer; many people would rather "take a pill" than make major dietary or lifestyle changes. Thus, many studies have been conducted in cell culture and animal models to determine the anticancer or cancer-preventive abilities of individual food components. In these models, many components of whole foods have been found to be detrimental to cancer cell growth or survival. These include the following examples (not a complete listing): n–3 FAs (6), dietary fiber (7), selenium (8,9), vitamin E (10,11), vitamin C (12), β-carotene (13,14), lycopene (15,16), melatonin (17,18), phytosterols (19,20), and sulforaphane (21,22).

A few very expensive clinical trials have tried to assess the effects of various purified dietary supplements on cancer development, with negative results (23). The lack of effects of dietary supplements on cancer risk could be due to one or more factors. For example, additive or synergistic interactions of components found in whole foods or in beneficial dietary patterns may provide the benefits against cancer seen in epidemiologic studies. An additional important consideration is that the development of human cancer is a long-term process. It is difficult to determine the exact timing during carcinogenesis at which nutritional supplementation would have an effect, the dose of supplement required, or the length of supplementation needed to reduce cancer risk. However, careful recent epidemiologic studies indicated that dietary patterns that include multiple beneficial foods can provide benefit against cancer and other chronic diseases (24–28).

Although foods may be the most appropriate unit to consider in relation to future health or disease, foods do consist of biochemicals. A specific food can be understood better by noting what biochemicals it contains, even if the isolated biochemicals, when consumed as supplements, do not decrease future disease. Walnuts are an example of 1 food that contains many components that have individually been found to be beneficial against cancer. Walnuts are an exceptional food that contains many components that have individually been found to be beneficial against cancer. Various studies have been initiated to test this hypothesis.

The purpose of an early study (30) was to determine whether or not walnut consumption had an effect on breast cancer growth. In this study, human MDA-MB 231 cells were implanted between the scapulae of athymic nude mice (n = 40 mice). The tumors were allowed to grow to 3 to 5 mm in diameter; the diet of half of the mice was then changed to include a human equivalent of 56.6 g of walnuts/d. The human equivalent of 56.6 g (2 ounces) of walnuts was calculated by determining the fraction of 2000 total calories/d that would be provided if 56.6 g/d of walnuts were consumed. That fraction, ~18% of calories, was then provided in the mouse diet. Diets were further balanced to account for the fat, protein, and fiber contained in the walnuts. The detailed compositions of the diets are provided in references 30 and 31. Twenty-two mice had growing tumors at the time of division into diet groups; thus, there were 11 mice per final diet group. The walnut diet was not started until after the tumors were measurable to identify the effect of the walnut on growth of the tumors, not on the ability of the cells to "take" and form a tumor.

**FIGURE 1** shows the growth rates of the tumors of mice fed either an AIN-76 rodent diet (32) modified to contain 10% corn oil as the dietary fat (CO diet) or the AIN-76 diet modified to contain walnuts with 10% total fat (30,31). Ten days were allowed after the initiation of the diets for the diet components to incorporate into the cells of the mice. Linear regressions of the

### TABLE 1 Cancer among Iranian women in Iran and Iranian migrants to British Columbia, Canada

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Ardabil province (Iran)</th>
<th>Kerman province (Iran)</th>
<th>Iranian migrants to British Columbia</th>
<th>British Columbia, general population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>7.6</td>
<td>16.9</td>
<td>68.5</td>
<td>81.4</td>
</tr>
<tr>
<td>Colorectal</td>
<td>Not done</td>
<td>5.9</td>
<td>11.6</td>
<td>26.6</td>
</tr>
</tbody>
</table>

1 Values are age-standardized incidences per 100,000 women. Original data were from Yavari et al. (68). Adapted from reference 3 with permission.
tumor growth rates from day 10 to day 35 after the start of the diets, followed by ANOVA of the growth rates, showed a significant suppression of the tumor growth rate as a result of walnuts in the diet \((P < 0.0001, t\) test). The rate of growth of the tumors in the group fed the walnut-containing diet was almost 80% slower than the growth rate of tumors in the group that was not fed the walnut diet.

Walnuts do not contain EPA \((20:5n-3)\) or DHA \((22:6n-3)\) (the 20- and 22-carbon metabolites of ALA); however, elongation and desaturation of the ALA resulted in significant increases in EPA and DHA in the livers of the mice fed the walnut-containing diet compared with those that were not fed the walnut diet \((P < 0.05)\). It was also reported that 1) the antioxidant capacity (Trolox equivalents) in serum and number of apoptotic figures in the tumors were slightly but not significantly higher, 2) proliferation in the tumor was significantly decreased \((P < 0.05)\), and 3) there was no difference in the mean body weight of mice fed the walnut diet compared with those that were not \((30)\).

Thus, it appeared that regular consumption of walnuts might slow the growth of existing tumors (or metastatic tumors after surgery or chemotherapy). However, many scientists believe that preventing cancer is a viable long-term strategy to reduce mortality from cancer \((33)\). Another study \((31)\) was initiated to determine if walnuts, consumed as part of a healthy diet, might reduce the risk of cancer development. In this study, a transgenic mouse genetically programmed to develop cancer was used to determine if walnut consumption could slow or prevent cancer development.

The C(3)1-TAg mouse was developed by Jeffrey Green as a model of breast and prostate cancer \((34,35)\). Mice carrying the transgene [a rat C3(1) prostatic steroid-specific promoter fused to the simian virus 40 large tumor antigen] develop mammary gland (females) or prostate (males) cancer with characteristics that resemble human cancers \((34,35)\). The experiment was designed to assess the effects of exposure to walnuts during gestation and lactation as well as lifelong exposure to walnuts. Such exposure would occur if walnuts were part of the usual diet of a population. Mature female mice were fed either corn oil \([CO\) diet \((the\) control diet)] or walnut-containing diets, then were mated with transgenic male mice. At weaning, the pups were randomly assigned to either the CO or the walnut diets, resulting in 4 final experimental groups. Groups are identified as dam’s diet/pup’s diet. For example, in the CO/walnut group, the dam’s diet was corn oil and the pup’s diet was the walnut-containing diet.

Figure 2 shows the results of this experiment \((31)\). It was found that the consumption of walnuts after weaning resulted in a 40% reduction in the tumor incidence (number of mice with any tumor), multiplicity (number of glands with a tumor per mouse), and median tumor mass. Lifelong exposure to walnuts resulted in a significant (~60%) reduction in these variables \((P < 0.05)\). In fact, only 40% of mice in the walnut/walnut group had tumors at 145 d, whereas 100% of mice exposed to the CO diet had mammary gland tumors. These results clearly show that dietary components can make a profound difference in carcinogenesis, even in the presence of an existing genetic mutation.

Other components of the walnut diet that could correlate with cancer reduction were also assessed. Of particular interest was whether or not the n-3 FAs contained in the diet would explain most of the reduction in cancer risk and tumor growth. To determine the contribution of ALA to tumor suppression, the results of a project to assess the ability of canola oil in the diet to suppress carcinogenesis were compared with the results of the walnut studies. The canola oil project used the same model and the same experimental design \((36)\), and the canola oil diet \([CA\)

![FIGURE 2](image-url) Tumor incidence (fraction of mice with a tumor) \((A)\), glands with tumor \((means \pm SE\)Ms) \((B)\), and total tumor mass \((individual\) tumors and group median) \((C)\) of mammary gland tumors in transgenic mice at 145 d of age fed diets without or with walnuts. Group names designate maternal diet/pup’s diet after weaning. For each graph, groups without a common letter differ \((P < 0.05)\). n = 10–13 mice/group. (Statistical analyses: tumor incidence, chi-square test; glands with tumor, ANOVA and Student-Newman-Keuls multiple-comparison test; tumor mass, Kruskal-Wallis and Dunn’s multiple comparison.) Reproduced from reference 31 with permission. CO/CO, corn oil diet (dam)/corn oil diet (pup); CO/Walnut, corn oil diet (dam)/walnut-containing diet (pup); Walnut/CO, walnut-containing diet (dam)/corn oil diet (pup); Walnut/Walnut, walnut-containing diet (dam)/walnut-containing diet (pup).
number of glands with tumors in the walnut/walnut mice was significantly less than in both the CA/CA and the CO/CO groups ($P < 0.05$). Thus, the ALA content of the walnut diet partially, but not completely, explained the reduction in tumors. A reasonable conclusion is that other components of walnut additively or synergistically contributed to tumor suppression. The finding that components in addition to ALA likely contributed to the cancer-suppression effects of walnuts is important because humans do not metabolize ALA to EPA and DHA as efficiently as do mice (37). Thus, if the effects of ALA were the only mechanism, the benefit would be less applicable to humans than to mice.

First, tocopherol amounts in the diets were calculated. The α-tocopherol content is not considered to have benefits against cancer (38) and may compete with γ-tocopherol, which does have benefits against cancer (11,39,40). It would be expected that the diet with the highest ratio of γ-tocopherol to α-tocopherol might have the most benefits against cancer. We calculated that the γ-tocopherol to α-tocopherol ratios were as follows: CO diet, 0; CA diet, 1.5; and walnut diet, 12.9. γ-Tocopherol has been shown to upregulate the activity of the PPAR in colon cancer (39) and prostate cancer cells (40). PPARs are also activated by PUFAs (41–43). Activated PPAR-γ signals antiproliferative, antiangiogenic, and prodifferentiation pathways in multiple tissue types (43). On the basis of these data it can be concluded that the γ-tocopherol content of the walnut diet could have added to the benefits of the ALA in reducing carcinogenesis.

Phytosterols, especially β-sitosterol (44,45), are thought to have cancer-chemopreventive potential. β-Sitosterol is found in many plant-based foods (46–49) and has been shown to induce first stage of cell proliferation cycle (G1) arrest (50) or apoptosis (51) in various cancer cell types. We calculated the β-sitosterol content of the diets to be as follows: CO diet, 968 mg/kg; CA diet, 413 mg/kg; and walnut diet, 71 mg/kg. Thus, the β-sitosterol content differences are not on the order that would correlate with the cancer incidences. However, this does not rule out that β-sitosterol contributed to the overall benefit of the walnut diet in combination with the other components.

Walnuts have pedunculagin, which is not contained in the other diets. Pedunculagin is formed from ellagic acid subunits that are metabolized to urolithins (52). Urolithins are thought to be the bioactive molecule of pedunculagin; they have been found to bind estrogen receptors (ERs) to inhibit breast cancer (52,53) and to be potent antioxidants (54–58). The presence of pedunculagin in the diet could have contributed to cancer suppression by walnuts. Cancer suppression due to ER-binding activity could have been especially important in the second study using the C(3) 1-TAg model because the tumors of this model start out ER-positive but progress to ER-negative (34), as do many human cancers. The data indicate that tumors were suppressed in the early stages. The antioxidant/antiproliferative activity of walnut-derived urolithins (56) could have been operational to suppress the growth of implanted ER-negative breast cancers in this tumor growth experiment.

Other studies have confirmed observations that walnuts provide benefits against breast and other cancers in mice. A mechanistic study showed that treatment with walnut extracts modifies multiple gene targets within MCF-7 human breast cancer cells and that both ALA and β-sitosterol slowed proliferation of these cells (59) and increased activation of the farnesoid X receptor (59). Activation of the farnesoid X receptor has been shown to induce apoptosis in breast cancer cells (60).

Walnuts have been shown to reduce prostate cancer growth in 2 models: a transgenic model (61) and an implanted tumor model (62). In the first study, suppression of prostate cancer growth was related to the suppression of insulin-like growth factor 1 (IGF-1). IGF-1 concentrations have been associated with risk of prostate and breast cancer (63) and could be an additional mechanism for the anticancer benefits of walnuts in the diet. In the second study, it was found that walnut-fed mice that did not develop tumors had less than one-half the hepatic $F_2$-isoprostane concentrations of the control mice that developed prostate tumors. $F_2$ isoprostane concentrations are a sensitive indicator of total oxidative load (64); thus, decreased concentrations of $F_2$ isoprostanes in the presence of a walnut diet would indicate decreased oxidative stress, perhaps due to the antioxidants in the walnut-containing diet.

Walnuts have also been shown to slow growth and angiogenesis in colon or renal cancer (65,66). In the first study (65), the growth of injected human colon cancer cells was compared in mice fed diets containing walnuts or flaxseed or corn oil. It was concluded that, compared with the CO diet, dietary walnuts significantly suppressed tumor growth by inhibiting angiogenesis. The second study (66) was a comprehensive study of the antiproliferative and antioxidant activities of walnuts against renal or colon cancer cell growth. Dietary phenols (e.g., pedunculagin or ellagic acid) are potent antioxidants (67). The total phenol contents of walnuts were extracted and applied at various doses to RBCs and renal or colon cancer cells. The extracts protected the RBCs from oxidative damage and showed a concentration-dependent inhibition of cancer cell growth (66). Given the evidence above, it seems likely that the addition of walnuts to a healthy diet could provide benefits against cancer and that these benefits are derived from multiple mechanisms.

The in vitro and in vivo studies to determine whether walnuts can help combat cancer are critical initial steps, but the important questions are whether this benefit can translate to humans and whether walnuts are beneficial as part of a usual diet. A pilot clinical trial has recently been initiated based on the hypothesis that if the walnut is modifying growth or other characteristics of breast cancer, then gene expression in the tumor should be modified. In this small study, next-generation RNA sequencing will be used to determine whether gene expression is modified by the consumption of 56.6 g of walnuts daily during the time between an initial biopsy (to diagnose the cancer) and surgery to remove the tumor. A modification of gene expression, similar to that seen in the animal and cell culture studies and which resulted
in slowed cancer growth, is expected. Results are yet to be obtained.

The above survey of available literature shows that walnuts have multiple ingredients that could act by multiple pathways to contribute to the suppression of the risk of developing cancer. It is likely that these ingredients act together to provide more benefit than would be expected from the individual components. We know that humans are not going to daily consume large amounts of walnuts, broccoli, fish, wine, or any other food that has been indicated to have benefit against cancer. However, scientists are demonstrating that these foods can reduce the risk of cancer or slow the growth of cancer, are identifying the bioactive components of these foods, and are uncovering the scientific mechanisms for the action of the food to determine which foods may act additively or synergistically. This base of knowledge is providing support for the notion that incorporating specific foods in a varied and healthy diet, adhering to an active lifestyle, and maintaining a healthy body weight can reduce the risk of cancer.

Acknowledgments
The sole author had responsibility for all parts of the manuscript.

Literature Cited

Walnuts for cancer prevention 5S of 6S


