ARE VITAMIN AND MINERAL DEFICIENCIES A MAJOR CANCER RISK?

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Diet is estimated to contribute to about one-third of preventable cancers — about the same amount as smoking. Inadequate intake of essential vitamins and minerals might explain the epidemiological findings that people who eat only small amounts of fruits and vegetables have an increased risk of developing cancer. Recent experimental evidence indicates that vitamin and mineral deficiencies can lead to DNA damage. Optimizing vitamin and mineral intake by encouraging dietary change, multivitamin and mineral supplements, and fortifying foods might therefore prevent cancer and other chronic diseases.

DEFICIENCY
Dietary intake of a vitamin or mineral at a level that is less than 50% of the recommended daily allowance — as distinguished from acute deficiency. For example, acute vitamin C deficiency causes scurvy.

RECOMMENDED DAILY ALLOWANCE
(RDA). The dietary-intake level that is sufficient to meet the daily nutrient requirements of nearly all healthy individuals in a defined group.

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Maximum health and lifespan require metabolic harmony. It is commonly thought that the problem of how to ensure adequate intake of the more than 40 essential micronutrients (vitamins, minerals and other biochemicals that are required in small amounts) has been solved for most of the world's population. Classical nutrient acute DEFICIENCY diseases such as scurvy, beri-beri, pernicious anaemia and rickets are no longer prevalent. Acute deficiencies of micronutrients are rare in developed countries, but suboptimal nutrient intake — less than the RECOMMENDED DAILY ALLOWANCE (RDA) — is a widespread problem (TABLE 1). Research indicates that considerable metabolic damage can still occur when nutrient intake levels fall below the RDA — even though they might not cause acute disease. For example, the optimum amount of folic acid or zinc that is required to minimize DNA damage and maximize a healthy lifespan is likely to be greater than the amount that is needed to prevent acute disease. Furthermore, the nutritional requirements of the elderly[2–4] are likely to differ from those of younger people, and have not been carefully examined. Nutritional requirements are also likely to depend on genotype[5].

Deficiencies in one aspect of the metabolic network can cause repercussions in many systems. A single deficiency, for example, can increase DNA damage (and cancer), promote neuronal decay (and cognitive dysfunction) or lead to mitochondrial disruption (accelerating ageing). The relationship between diet and cancer has, historically, been thought of in terms of exposure to potential carcinogens, such as alcohol or heterocyclic amines. Dietary deficiencies, however, might be a much more important factor in cancer risk. Evidence for a link between various micronutrient deficiencies and DNA damage has been accumulating in recent years[6–9], but this has been difficult to study and, as a result, has not been the main focus for epidemiology researchers[10].

The importance of nutrition in cancer development is actively studied and is controversial. Several studies have examined the association between diet and specific cancers, including breast[11,12], prostate[13] and colorectal cancer[14]. The associations between cancer and specific dietary factors, such as meat, fruits and vegetables, and specific nutrients, such as vitamin D and selenium, have been established[15–18]. The most convincing epidemiological evidence for the role of dietary factors in cancer risk is the inverse relationship between the consumption of fruits and vegetables and many types of cancers. Comprehensive reviews have shown that people who consume the fewest fruits and vegetables, compared to those who consume the most, have a higher cancer incidence[16,19–21]. Most CASE–CONTROL STUDIES

Summary

- Acute deficiencies of vitamins and minerals are rare in developed countries, but suboptimal nutrient intake — less than the recommended daily allowance (RDA) — is a widespread problem. Research indicates that considerable metabolic damage can still occur when nutrient intake levels fall below the RDA — even though they might not cause acute disease.
- Evidence indicates that deficiencies of iron and zinc, and the vitamins folate, B12, B6 and C, can cause DNA damage and lead to cancer.
- New animal bioassays of nutritional deficiencies are needed, particularly for studying cancer.
- Reduced folate intake has been associated with cancer. Folate, B6 and B12 deficiencies cause the incorporation of deoxyuracil into DNA, leading to DNA breakage, and could promote tumorigenesis.
- The relationship of vitamin and mineral deficiencies and cancer is extremely complex. An integrated analysis of the findings from epidemiological, animal-model, metabolic and intervention studies, as well as from genetic polymorphism research, is required.
- Approaches to eliminating micronutrient deficiencies include improving diet, fortifying foods and providing multivitamin and mineral supplements. Prevention strategies such as these could have a significant impact on cancer and public health, with minimal risk being involved.

indicate that a reduced consumption of fruits and vegetables can double the risk of developing most types of cancer[16,19–21] (TABLE 2). In 1992, a comprehensive review by Block and colleagues[19] found that 75% of nutritional studies reported a significant association between cancer protection and the consumption of fruits and vegetables. A more recent review (which did not include cohort studies) reported a similar result, stating that 77% of nutritional studies associated fruit and vegetable consumption with cancer protection (TABLE 2). It should be noted that, for breast and colon cancer, some large-scale PROSPECTIVE COHORT STUDIES and recent case–control studies have failed to show a link with low levels of fruit and vegetable consumption[22–25].

Whole grains are a better source of some vitamins or minerals (such as vitamin B6 and magnesium) than fruits and vegetables. Increased consumption of whole grains has also been associated with a decreased risk of several cancers[26,27]. A META-ANALYSIS of 40 case–control studies showed that whole grains were associated with a 40% decreased risk of developing stomach cancer and a 20% decreased risk of developing cancers of the rectum and colon[27]. Although epidemiological studies indicate an overall higher cancer risk in people who consume the fewest fruits and vegetables or the fewest whole grains (TABLE 2), they have not, however, established a causal link between consumption of these foods and cancer. Also, it is not known which of the many dietary constituents in fruits and vegetables or whole grains are responsible for, or the cellular or molecular processes that confer, the protective effects. It is worth noting that meat — the main food source of iron, zinc and B12 — is also an important source of micronutrients (TABLE 3).

The mechanisms of action of dietary micronutrients are complex and are not fully understood. Micronutrients might function as antioxidants, anti-mitogens, anti-mutagens or in other ways[28–31]. Impending research by both bench scientists and epidemiologists on the many factors that are involved in the diet–cancer relationship, including gene–environment interactions, should begin to clarify the complex relationship between diet and cancer[32]. Though DNA damage is a well-established risk factor for cancer causation, it should be emphasized that cell-division rates and other factors also contribute[33–37].

Establishing a link between micronutrient intake, DNA damage and cancer is only one important area of research on diet and cancer, but it lends itself to an inexpensive and practical solution. 'Tuning up' metabolism could reduce the incidence of cancer, as well as have other health benefits, at little cost — a full year's supply of daily multivitamin and mineral pills for one person costs less than a few packs of cigarettes. In this article, we focus on cancer protection that is conferred by vitamins such as folate, B12, B6 and C, as well as the minerals iron and zinc (TABLE 3).

CASE–CONTROL STUDY
An epidemiological study design in which individuals are selected based on the presence (case) or absence (control) of disease. Well-designed case–control studies require that the two groups are derived from the same population.

PROSPECTIVE COHORT STUDY
An epidemiological study design in which individuals with known characteristics (such as occupational exposure, smoking and exercise) are enrolled and followed over time for specific outcomes. The rate of cancer (or other disease) in the exposed population is compared to that in the unexposed population.

META-ANALYSIS
A retrospective analysis of the results from different studies, making certain assumptions, to reach a conclusion that is based on the pooled data.

Table 1 | **Micronutrient deficiencies in US individuals**

Nutrient	Population group	Current RDA	% Consuming less than the RDA	% Consuming less than half the RDA
Minerals				
Iron	Women 20–30 years Women 50+ years	18 mg 8 mg	75% 25%	25% 5%
Zinc	Women/men 50+ years	8/11 mg	50%	10%
Vitamins				
Folate*	Women 20+ years Men 20+ years	400 µg 400 µg	75% 75%	50% 25%
B6	Women/men 20+ years	1.5/1.7 mg	50%	10%
B12	Women 20+ years Men 20+ years	2.4 µg 2.4 µg	25% 10%	10% 5%
C	Women/men 20+ years	75/90 mg	50%	25%

*Folate intake before US fortification in 1998. RDA, recommended daily allowance. Data adapted from REF. 143; dietary intakes include food fortification, but not supplement use.

Table 2 | **Epidemiological studies showing cancer protection from fruits and vegetables**

Cancer site	1992 review: fraction of studies showing significant cancer protection[19]	1992 review: relative risk (median) (low versus high quartile of consumption)[19]	1997 review: fraction of studies showing significant cancer protection[16]
Epithelial			
Lung	24/25	2.2	11/13
Oral	9/9	2.0	13/15
Larynx	4/4	2.3	6/8
Oesophagus	15/16	2.0	15/18
Stomach	17/19	2.5	28/31
Pancreas	9/11	2.8	9/11
Cervix	7/8	2.0	4/6
Bladder	3/5	2.1	6/8
Colorectal	20/35	1.9	3/6
Colon	15/21	–	–
Miscellaneous	6/8	–	–
Hormone dependent			
Breast	8/14	1.3	8/12
Ovary/endometrium	3/4	1.8	7/9
Prostate	4/14	1.3	1/6
Total	**129/172 (75%)**		**126/164 (77%)**

Challenges to nutrition research

Epidemiological analysis. Epidemiological studies investigate the link between exposure to one or more variables, and a defined outcome, such as the incidence of particular cancers in a carefully described population. The exposure can be a nutritional variable such as dietary pattern, consumption of an individual food, nutrients or non-nutrient components of foods, or chemical alterations that occur during cooking or preservation. Diet is therefore very complex to measure.

These complexities are multiplied when researchers attempt to measure components of foods such as micronutrients. Micronutrient status can be measured by markers in blood, urine or tissue, although the use of biological markers to measure micronutrient status has its own limitations. Nutrients are never isolated alone, and the presence of one is usually associated with the presence of another. For example, β-carotene and vitamin C are markers for fruit and vegetable intake. Studies that report on the effects of a specific nutrient should therefore always be viewed cautiously.

Another complication in forming associations between diet and cancer is that the investigator must make an *a priori* decision about the pertinent exposure time, such as whether to study the cumulative exposure over time, average exposure over time or peak exposure at a crucial time, such as the first trimester of pregnancy. There are also CONFOUNDING FACTORS that must be considered when making associations with diet, such as sociodemographic factors, as well as absorption, bioavailability, transport distribution and measurement of a particular nutrient[38]. For example, phytic acid (inositol hexaphosphate), which is found at high levels in cereal grains and legumes, forms a tight complex with zinc or iron that decreases absorption. Circulating levels of nutrients also depend on transport proteins or other co-enzymes, and marker analysis can be affected by personal habits, such as smoking, drug use or dietary factors. Aspects of epidemiological study design can also affect results (BOX 1).

Because it is so difficult to determine the exact levels of micronutrient intake in a person's diet, the most direct approach to assaying nutrient effects would be to perform supplementation studies. Smaller clinical or metabolic studies of subpopulations of individuals with identified genetic alterations might be more valuable than large epidemiological studies that include several genotypes. INTERVENTION STUDIES that measure chromosome breaks or other DNA damage in small numbers of people with a low intake of a micronutrient before, during and after supplementation with the micronutrient might be the most successful research approach.

Patterson *et al.*[28] published a comprehensive review of epidemiological studies from 1980 to 2000, summarizing the results of randomized, controlled cancer trials that assessed the association between intake of micronutrient supplements and cancer. Although some individual studies of micronutrient supplement intake have associated nutrient supplements with lower cancer risk, the authors concluded that there is not sufficient high-quality data on which to base firm conclusions.

Experimental models. In model systems that attempt to correlate diet with cancer risk, there is always uncertainty as to whether results obtained from the

CONFOUNDING FACTOR
These occur because behaviour-related variables of interest tend to cluster. An exposure (for example, vegetable consumption) might be of interest in protecting against a particular cancer. However, if smokers eat fewer vegetables than non-smokers, we might falsely attribute a risk reduction to vegetables that is really owing to the fact that a higher proportion of vegetable-eaters are non-smokers. Confounding factors can be controlled for by separating the smokers and the non-smokers and asking whether the vegetable–cancer association is seen in both groups, or by more sophisticated, but conceptually similar, statistical techniques.

INTERVENTION STUDY
Often called a clinical trial or experimental study, an epidemiological analysis of a hypothesized cause–effect relationship that is performed by modifying a supposed causal factor, such as lack of vitamin C consumption, in a population.

Table 3 | **Micronutrient sources and main contributing foods in the Western diet**

Nutrient	Richest food sources	Primary sources in US diet
Folate	Fruits and vegetables, including dark greens and dried beans	Fortified cold cereal*, orange or grapefruit juice, green salad, fibre or bran cereals , white bread‡
Vitamin B12	Meat, fish, milk products, fortified cereals	Beef, fortified cold cereal*, shellfish, low-fat milk
Vitamin B6	Fortified cereals, whole grains, meat	Fortified cold cereal*, white potatoes, bananas, chicken or turkey, beef
Vitamin C	Citrus fruits and vegetables	Orange or grapefruit juice, drinks/juices with vitamin C, other fruit
Iron	Meat	Cold cereal‡, fibre or bran, white bread‡, rolls‡, buns‡, bagels‡, pizza‡, hamburgers, other beef
Zinc	Meat, eggs, nuts	Beef, fortified cold cereal*, cheese or cheese spread, mixed dishes with beef/pork/veal/lamb

*Fortified with essential vitamins and minerals. ‡Enriched/fortified with iron, riboflavin, thiamine and niacin. Data adapted from REF. 144.

experimental system accurately reflect processes that occur in tissues or whole organisms. Some studies have used animal models to address the role of dietary factors in causing or preventing cancer, leading to hypotheses about physiological mechanisms and predictions about dose–response relationships between dietary compounds and cancer risk. However, there are limitations to extrapolating the findings of animal studies to humans, owing, in part, to differences in metabolic pathways, rates and lifespans. In some cases, there are known differences between species in the metabolism of vitamins. Vitamin C, for example, is synthesized by rats and mice, but must be provided in the diets of guinea pigs and humans.

Relatively few animal studies have examined the link between vitamin or mineral deficiency and cancer. Better animal models are needed to elucidate mechanisms that are related to cancer prevention. This might be a more effective strategy than testing the effect of exposure to high doses of synthetic chemicals, when humans are typically exposed to only low doses[39,40].

Folate

Our current understanding of folate deficiency and its relationship to cancer illustrates the importance of considering the findings of all types of research — epidemiological, molecular, clinical and interventional — when investigating the link between diet and cancer. There is much epidemiological evidence indicating that low folate intake increases the risk of many types of cancer (TABLE 4). As shown in TABLE 4, reduced folate intake has been associated with a higher risk of colon cancer[14,41], and long-term use of a folate-containing supplement has been shown to lower the risk of colon cancer by 75%[42]. There is also evidence that low folate intake increases the risk of breast cancer[43,44], pancreatic cancer in smokers[45,46], and gastric and oesophageal cancers[47].

In vitro studies have shown that folic-acid deficiency causes a dose-dependent increase in uracil incorporation into human lymphocyte DNA[48] (FIG. 1). Folate administration reduces DNA uracil incorporation and the occurrence of chromosome breaks in human cells[7]. *Ex vivo* experiments have shown that all the markers of chromosome damage in human

Box 1 | **Limitations to specific types of epidemiological studies**

A number of factors make it especially complex to perform epidemiological studies to associate diet and disease. In case–control studies, participants are susceptible to RECALL BIAS. Prospective cohort studies might not cover the period of time during which subjects experienced a micronutrient deficiency that was crucial for the development of cancer. For example, the predisposition to cancer might have occurred during germ-cell or fetal development, or during childhood. Study duration might not also have had sufficient follow-up time for cancers to be manifest.

Most epidemiological studies use questionnaires to measure dietary intake, rather than assaying micronutrient levels directly. In carefully designed studies, nutrient levels in the blood are analysed in combination with dietary data. One of the primary reasons that epidemiological studies are sometimes inconclusive is that conclusions are drawn from data that might contain measurement error in estimating micronutrient intake. Dietary questionnaires must be carefully designed to cover all appropriate foods, nutrients and supplements. Nutrient estimates are derived from databases or food-composition data tables and must be updated to reflect the ever-changing food supply, and to provide an accurate assessment of nutrient intake. Another important methodological issue is that trials should carefully monitor dose and duration of use of supplements, measurement of long-term micronutrient intake, and other aspects of a healthy lifestyle.

A final important methodological problem in performing epidemiological analyses of micronutrient intervention and disease is that data from most people, who usually consume saturation levels of a particular micronutrient, are combined with those of the smaller percentage of people (generally 10–20%), who have an inadequate intake, which makes it easier to miss an association. Careful design and appropriate analyses of data are required to help overcome some of these challenges.

RECALL BIAS
Occurs in individuals that describe events (such as exposures, diseases and pregnancy outcome) of the past in a non-comparable manner. It is primarily a problem in case–control studies when that presence of the disease in one group might result in differential recall (for example, of alcohol consumption or dietary behaviour) between the cases and controls.

Table 4 | **Evidence for folate, B6 and B12 deficiency and cancer risk**

Type of cancer	Comments	References
Colorectal cancer, adenomas	Lower intake of folate was associated with higher risk of colon cancer; long-term use of folate supplement lowers the risk of colon cancer by 75%	14,41,42
	No association was seen between folate, B6 and B12 and risk of colorectal hyperplastic polyps	146
Breast cancer	Strong inverse association was seen between folate intake and risk of breast cancer among women who drink alcohol, which interferes with folate absorption	43,44
	No association was seen between folate and B6 and breast cancer	67
Pancreatic cancer	Risk of pancreatic cancer in smokers was inversely associated with dietary folate	45,46
Oesophageal and gastric cancers	Folate and vitamin B6 were inversely associated and vitamin B12 was positively associated with these cancers in a case–control study	47
Acute lymphoblastic leukaemia (ALL; children)	ALL was associated with the *MTHFR* genotypes, indicating a role of folate in the development of ALL	59
Acute lymphocytic leukaemia (ALL; adults)	A significant reduction in risk of ALL was found in those with the *MTHFR 677TT* genotype, indicating that folate deficiency might be a risk factor	58
Cervical cancer	Risk of invasive cervical cancer was elevated for women with higher serum homocysteine	70
	Dietary intakes of folate, B6 and B12 were inversely related to the risk of developing cervical dysplasia	60
	No statistically significant association between folate, B12 and cervical cancer was found	66
Prostate cancer	Lower intake of B6 was associated with prostate cancer	68
Lung cancer	Lower intake of B6 (higher B6 serum level) was associated with lung cancer	69

lymphocytes are minimized at folic-acid concentrations that are higher than the RDA[48–50]. Folate supplementation above the RDA has also been shown to reduce chromosome breakage in humans[49,50]. Although this study did not examine cancer incidence, chromosome breaks have been linked to cancer in other studies[51].

A clue to the folate–cancer connection was the discovery of a polymorphism (*C677T*) in the gene that encodes methylene-THF reductase (MTHFR) — the enzyme that reduces methylene-tetrahydrafolate (CH_2=THF) to methyl THF (FIG. 1). This polymorphism decreases the activity of MTHFR, which increases the methylene-THF pool at the expense of the methyl-THF pool, resulting in decreased incorporation of uracil into DNA and an increased number of chromosome breaks. It is a common polymorphism in populations of people who live in northern regions of the world, with 5–25% of individuals being homozygous for this polymorphism and up to 50% being heterozygous.

Several studies have shown a two- to fourfold lower risk of colon cancer in individuals who are homozygous for the *677T* allele of methylene-THF reductase (*MTHFR-TT*), compared with individuals who are homozygous for the *C677* allele and have a high folate intake[52–55]. At low folate levels, however, the *MTHFR-TT* genotype does not seem to be protective and might even be a risk factor. Other studies on adenomatous polyps show an increased risk for developing the *MTHFR-TT* genotype[53,56,57]. Adult acute lymphocytic leukaemia (ALL)[58] and childhood ALL[59] have also been inversely associated with the *MTHFR-TT* genotype, indicating that folate deficiency might promote ALL.

Unfortunately, the *MTHFR-TT* genotype is not all good news. As the lower level of MTHFR decreases the methyl-THF pool, it increases serum levels of homocysteine — a risk factor for endothelial-cell damage and cardiovascular disease[32]. Individuals who are homozygous for this allele have a twofold increase in plasma homocysteine levels.

Low dietary intake of folate, B12 or B6 has been associated with a higher risk for developing cervical squamous epithelial lesions in a case–control study[60]. In contrast to studies mentioned above, individuals with the *MTHFR-TT* genotype also had a higher incidence of cervical cancer. The authors suggest this is due to a lack of inhibitory methylation — that results from a decreased methyl-THF pool — of human papillomavirus (HPV), which is a significant cause of cervical cancer[60].

Why is this polymorphism so frequent in populations in the northern United States? Although it is credible that these populations were, historically, chronically folate deficient because of their diet, cancer and heart disease come too late in life to select for individuals with a lower risk. The *MTHFR-TT* genotype might be selected for based on its ability to reduce uracil incorporation into sperm DNA, which consequently results in less DNA damage in offspring[6,61]. Levels of non-methyl THFs are positively associated with sperm count and density[61]. Folate levels are therefore also important for male reproductive function, and further support the concept that folate deficiency can cause DNA damage. Germ-line damage to the sperm has also been linked to childhood cancers[62]. Further studies are required to determine if ALL is higher in children whose fathers have a poor diet[59].

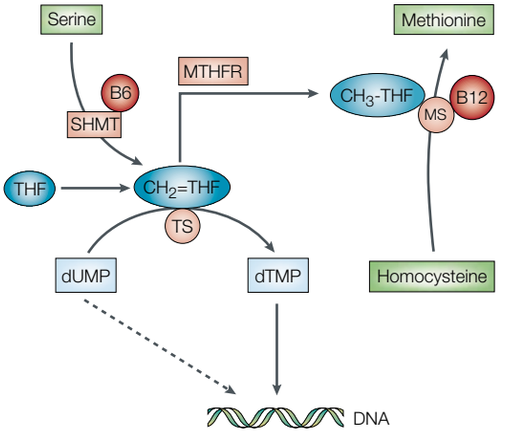

Figure 1 | **Incorporation of uracil in DNA.** Folate (THF), B6 or B12 deficiencies have all been associated with cancer. Cellular depletion of any of these vitamins can induce DNA damage by causing deoxyuridine (dUTP), instead of deoxythymidine (dTTP), to be incorporated into DNA. Methylene-THF reductase (MTHFR) converts methylene-THF (CH_2=THF) to methyl-THF (CH_3-THF). The CH_2=THF pool is derived from folate and is required for the methylation of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) by thymidylate synthetase (TS). Folate deficiency decreases the amount of CH_2=THF, causing dUTP to accumulate in DNA (dashed line), rather than dTTP, which results in chromosome breaks[7]. B6 deficiencies decrease the activity of the enzyme serine hydroxymethyl transferase (SHMT), which is required to produce CH_2=THF. The resulting smaller CH_2=THF pool results in dUTP incorporation into DNA. Methionine synthetase (MS), a B12 and CH_3-THF-dependent enzyme, converts homocysteine to methionine. When B12 is deficient, THF is trapped as CH_3-THF. This reduces the size of the CH_2=THF pool, leading to increased dUTP incorporation into DNA. When dUTP is incorporated into DNA, it is normally excised by a glycosylase repair enzyme, which induces transient single-strand breaks in the DNA. Two opposing single-strand breaks cause a double-strand chromosome break. Double-strand breaks are difficult for cells to repair, and increase cancer risk[51].

Vitamins B12 and B6

Vitamin B12 deficiency would be expected to cause chromosome breaks by the same uracil-misincorporation mechanism that is found with folate deficiency[32]. Both B12 and methyl-THF are required for the methylation of homocysteine to methionine (FIG. 1). If cells are deficient in either folate or B12, homocysteine accumulates. When B12 is deficient, tetrahydrofolate is trapped as methyl-THF, reducing the methylene-THF pool, which is required for methylation of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP). B12 deficiency, like folate deficiency, therefore causes uracil to accumulate in DNA (B. N. A., unpublished observations). In a study of healthy elderly men[63] and young adults, increased chromosome breakage was associated with low dietary intake of either B12 or folate, or with elevated levels of homocysteine[49,63,64]. B12 supplementation above the RDA was necessary to minimize chromosome breakage[49,50].

Vitamin B6 deficiency would also be expected to result in uracil misincorporation. B6 deficiency causes a decrease in the enzyme activity of serine hydroxymethyl transferase, which supplies the methylene group for methylene-THF[65] (FIG. 1). So the methylene-THF pool is decreased in instances of B6 deficiency, leading to uracil incorporation and subsequent chromosome breaks (B. N. A., T. Shulz and S. Mashiyama, unpublished observations). Several studies have shown that, in individuals with the *MTHFR-TT* genotype, vitamin B6 intake is associated with protection against colon cancer and/or adenomas[53,55,57].

Recent epidemiological studies have indicated an association between B6 or B12 deficiency and cancer prevention, but results are mixed[47, 66, 67]. In one case–control study of diet and cancer, vitamin B6 deficiency was associated with prostate cancer[68]. A significantly lower risk of lung cancer was found in men with higher serum B6 levels[69]. Compared to men with the lowest vitamin B6 concentrations, men in the highest quintile had about one-half the risk of lung cancer. No associations were made between B12, folate or homocysteine and lung cancer. Serum homocysteine levels were found to predict the risk of developing invasive cervical cancer in a large case–control study[70].

Oxidation, micronutrients and cancer risk

Oxidants, such as radiation, are known mutagens, so antioxidant micronutrients, such as vitamins C and E, might function as anti-mutagens and anti-carcinogens[71,72]. The evidence that supplementation with these vitamins lowers cancer risk is inconclusive.

Vitamin C. Both experimental and epidemiological data indicate that vitamin C protects against stomach cancer[47,71,73]. This is a plausible conclusion, as oxidative damage from inflammation caused by *Helicobacter pylori* infection is a risk factor for stomach cancer[74]. Fruit and vegetable intake — the main dietary source of vitamin C — is also inversely associated with stomach cancer (TABLE 1). Mayne and colleagues have also reported an inverse association between vitamin C and oesophageal adenocarcinoma[47].

Many other studies, however, have reported no effect of vitamin C on cancer risk. A thorough review of intervention studies showed both positive[14] and negative[12] studies, and so the evidence is inconclusive[75]. There are several reasons why a positive effect might not have been observed. The blood-cell saturation of vitamin C occurs at about 100 mg/day in humans[76]. Evidence indicates that this level minimizes DNA damage[9,77–79]. Perhaps the differing results from various studies were caused by differences in whether vitamin C reached tissue saturation levels in the population that was studied. If only a small proportion of the population had inadequate tissue saturation by vitamin C intake, a real effect would be missed (TABLE 1). Other factors that might explain the difference in results include failure to adequately assess vitamin C intake, failure to assess

Box 2 | **Other nutritional factors linked to cancer**

Evidence continues to accumulate to support the importance of several other micronutrients, such as vitamin D, calcium, niacin and selenium, in cancer development. Epidemiological findings, although not entirely consistent, indicate that there is a relationship between vitamin D deficiency and several types of cancer, primarily colorectal cancer and colorectal adenomas[18,127]. Vitamin D is a hormone that is synthesized in skin that has been exposed to ultraviolet light. Vitamin D deficiency increases cell proliferation, and so is a risk factor for cancer[128]. Populations in northern regions of the world are chronically vitamin D deficient unless they drink fortified milk. People with darker skin in northern regions of the world who don't drink fortified milk (dark skin is associated with lactose intolerance) are at the greatest risk for vitamin D deficiency and cancer.

Several studies have reported a weak association between increased calcium intake and decreased risk of colorectal cancer, but high calcium intakes (above the recommended daily allowance (RDA)) have also been associated with an increased risk of prostate cancer[129,130]. More research is needed to clarify the role of calcium, vitamin D and related factors in colorectal and prostate cancer.

Epidemiological studies, including cross-sectional, case–control and prospective studies, support the inverse association between serum selenium levels and lung, colorectal and prostate cancer in men[131–133]. Several selenocysteine-containing proteins are part of the antioxidant defence network. The evidence for selenium has been felt to be sufficient to justify a large clinical trial on prostate cancer[134]. In a large international case–control study, an increase in niacin — one of the B vitamins — along with antioxidant nutrients were found to be associated with a decrease in oral cancer (the mouth, pharynx and oesophagus)[135,136]. Niacin is a component of NAD, which is involved in the polyADPribose defence against DNA strand breaks.

Other nutritional factors that are implicated in cancer include obesity/type II diabetes, alcohol, fat, lack of fibre and phytochemicals[12,137]. Research on obesity and cancer is an active area; it includes research that examines not only body-mass index, but also factors that are intimately related to obesity and being overweight, such as physical activity and the hormone insulin[138–141]. The relationships between fibre, fat, phytochemicals and cancer have been extensively reviewed[16,142], but findings are not consistent. The effect of fibre is difficult to separate from other highly correlated dietary variables in whole grains, fruits and vegetables, whereas the effect of fat is difficult to separate from calorie intake.

whether people were using vitamin supplements or failure to take into account the effect of modifiers such as body-mass index or smoking (which decreases plasma vitamin C levels)[80].

Many studies have investigated the effects of vitamin C supplementation in humans using biomarkers of oxidative damage to DNA, lipids (lipid oxidation releases mutagenic aldehydes) and protein[81–85]. For example, intervention studies with antioxidant supplements (100 mg per day of vitamin C, 28 mg per day of vitamin E and 25 mg per day of β-carotene) were found to decrease DNA strand breaks in lymphocytes, as measured by the COMET ASSAY[77]. Subsequent studies showed β-carotene by itself was ineffective at reducing the number of DNA breaks[86], but that vitamin C was effective alone[87].

Studies in rats have shown that the spontaneous oxidative damage occurs at a rate of about 66,000 DNA adducts per diploid cell[88,89] and, unlike uracil misincorporation, is likely to occur with equal frequency on both strands. Repair of oxidative adducts by glycosylase results in transient single-strand breaks in DNA. Increased oxidative damage is therefore associated with low vitamin C intake[9]. Individuals who are deficient in both folate and antioxidant intake would have higher levels of both oxidative damage and of uracil incorporation in their DNA, and be expected to have a high level of double-strand DNA breakage. Radiation (an oxidative mutagen) and folate deficiency have been shown to act synergistically in causing chromosome breakage in tissue-culture cells[90].

Smoking is another producer of oxidative stress. A smoker needs to consume 40% more vitamin C than a non-smoker in order to maintain a comparable blood-plasma level of vitamin C[91]. Several studies have examined the associations between paternal smoking, vitamin C intake, oxidative damage to sperm DNA and childhood cancer in the offspring. Smoking depletes vitamin C, which is required to protect DNA in sperm against oxidative damage[9,92,93]. Smokers, or men with low ascorbate intake, have lower seminal-plasma ascorbic-acid levels and higher levels of oxidative DNA damage in their sperm than either non-smokers or men with adequate ascorbate intake[9,92].

Unfortunately, there have been few studies that have rigorously examined the effect of paternal vitamin C level by itself on cancer in offspring. Nevertheless, there is evidence that children with fathers who smoke have an increased rate of childhood cancer[94–97]. An epidemiological study from China makes a particularly strong case that ALL, lymphoma and brain cancer are each increased three- to fourfold in children of male smokers[97]. The associations were strongest in men with the highest number of PACK YEARS of smoking. It seems likely, given the available evidence, that the cancer risk to offspring of male smokers would be higher when dietary antioxidant intake is low.

Other micronutrients in addition to iron and zinc, which are discussed below, have antioxidant potential. These include carotenoids, vitamin E and selenium. These have been the focus of experimental and epidemiological research to determine the association with cancer risk[16] (BOX 2).

COMET ASSAY
A technique that uses electrophoresis of immobilized single cells to measure DNA strand breaks.

Iron. The epidemiological data on iron and cancer are mainly limited to studies of iron excess. Excessive iron has long been known to catalyse oxidation *in vitro*. Increased risk of human cancer is associated with excess iron[98,99]. The increased risk of hepatic carcinoma in individuals with cirrhosis caused by HAEMOCHROMATOSIS indicates a link between iron overload and cancer. Several epidemiological studies have reported associations between increased iron status and colorectal cancer. A recent review of 26 publications on iron and colorectal cancer risk found that approximately three-quarters of the larger studies supported the association of excess iron with colorectal cancer risk[100]. Excess iron also seems to lead to oxidative DNA damage in rats, which is reversed by vitamin E[101].

But iron deficiency, as well as iron excess, leads to oxidative DNA damage[102]. Iron deficiency is one of the most common micronutrient deficiencies — it affects 2 billion women and children worldwide, and about 25% of menstruating women in the United States alone (TABLE 1). How does iron deficiency cause oxidative damage? One mechanism involves haem deficiency (haem uncouples mitochondria and causes oxidant release and mitochondrial DNA damage), as well as the loss of some important iron-containing defence enzymes, such as catalase and haem oxygenase II[103]. Data from epidemiological studies that link iron deficiency to cancer incidence, however, are lacking.

Zinc. Intake of the trace element zinc is below the amount that is considered to be adequate or desirable in many populations[104] (TABLE 1). Zinc is found in all body tissues and is one of the most abundant intracellular elements. Acute zinc deficiency is not easily achieved in adult humans[105], but when it does occur it causes various health effects[104,106,107]. Zinc is a component of more than a thousand DNA-binding proteins that contain zinc fingers, as well as copper-zinc superoxide dismutase, the oestrogen receptor and synaptic transmission proteins[106]. The *TP53* gene, which encodes p53 in humans, is mutated in half of human tumours, and loss of zinc binding disrupts its ability to mediate the DNA-damage response[108,109,110] (E. Ho and B. N. A., unpublished observations). Zinc deficiency also causes loss of function of zinc-containing DNA-repair enzymes[110], thereby compromising the ability of the cell to repair the damage and so promoting tumorigenesis.

Rats placed on a zinc-deficient diet and the offspring of zinc-deficient rhesus monkeys both have a higher incidence of chromosome breaks[111,112]. These chromosome breaks seem to be caused by oxidative damage[111,113], which could result from a loss of activity of copper-zinc superoxide dismutase or formamidopyrimidine glycosylase — a zinc-containing DNA-repair enzyme that repairs oxidized guanine[114].

Zinc deficiency might also contribute to oesophageal cancer in humans. In conjunction with a single low dose of a chemical carcinogen (nitrosamine), it has been shown to cause oesophageal tumours in rats[115–117], and zinc deficiency alone has also been shown to cause this cancer[115]. Replenishment of zinc in zinc-deficient rats can induce apoptosis in oesophageal epithelial cells, which could reduce the risk of oesophageal cancer[118].

Zinc is essential for testicular development and spermatogenesis[119]. Zinc concentration is hundreds of times greater in seminal plasma than in blood plasma, and it is believed to be important for spermatogenesis and maintaining the stability of spermatozoa[106]. Zinc concentrations in blood and seminal plasma are correlated positively with sperm-cell density, and lower zinc concentrations are found in infertile men compared with fertile men[120]. Combined zinc and folate supplementation increased the total normal sperm count in subfertile men in a randomized intervention study, although an independent effect of the two nutrients was not seen[121]. Zinc deficiency leads to increased oxidative damage to testicular-cell DNA (as measured by oxo^{8}dG); this might also contribute to childhood cancer[113].

Conclusions and future directions

More than 40 micronutrients are required in the human diet. The effects of deficiencies of these micronutrients, both individually and in combination, often leads to DNA damage, which can lead to cancer. These effects can be studied in human cell cultures, and followed-up through intervention studies in people that have dietary deficiencies in specific micronutrients. Another active area of nutrition research has been the study of polymorphisms in genes that encode metabolic enzymes, and how these affect cell processing of particular micronutrients. This research is useful in establishing causality between micronutrient deficiencies and cancer[32,58–60].

Micronutrient deficiencies are relatively common, but can be easily remedied. Approaches to eliminating micronutrient deficiencies include improving diet, fortifying foods and encouraging multivitamin and mineral supplements that contain the RDA levels. Cancer-prevention strategies such as these could have a significant impact on public health, with minimal risk being involved[122–124].

Though consumption of the RDA levels of micronutrients is of minimal risk, some people take too many supplements. Mae West's quote "too much of a good thing is wonderful" does not apply to nutritional supplements. Too much of a mineral or even a vitamin can be toxic. The RDA committees are now listing upper limits (UL) for minerals and vitamins. Cancer-intervention trials of β-carotene, which involved doses that were well above those that would be obtained from a normal diet, had a deleterious effect, reinforcing the importance of exceeding the RDA of micronutrients[125,126].

People who eat very few fruits and vegetables are likely to have an inadequate intake of many micronutrients, such as folic acid and vitamin C, which contributes to DNA damage, cancer and other degenerative diseases. In addition, dietary deficiencies of micronutrients that are not derived primarily from

PACK YEARS
The number of years of tobacco use, multiplied by the number of packs per day. For example, 1 pack year is 20 cigarettes per day for 1 year, 40 cigarettes per day equals 2 pack years.

HAEMOCHROMATOSIS
A genetic disorder and the most common form of iron overload disease, which is characterized by iron deposition in the liver and other tissues as a result of a small increase in intestinal iron absorption over many years. It most often affects white northern Europeans: 1 in 8–12 is a carrier of the abnormal gene, and men are five times more likely to be diagnosed with haemochromotosis than women.

fruits and vegetables, such as zinc, iron, and the vitamins E, niacin, B6 and B12, also seem to contribute to DNA damage. We anticipate that other micronutrients will be added to this list in the coming years. With more research, we will develop a better understanding of the mechanisms by which specific micronutrients regulate normal cell function, and how their deficiencies can alter normal metabolism. 'Tuning-up' human metabolism, which varies with genetic constitution and changes with age, could prove to be a simple and inexpensive way to minimize DNA damage, prevent cancer, improve health and prolong a healthy lifespan[2–4,145].

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GenBank: http://www.ncbi.nih.gov/Genbank/
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