BIOCHEMICAL AND MOLECULAR MEDICINE 54, 1–11 (1995)

REVIEW

Proposed Role for a Combination of Citric Acid and Ascorbic Acid in the Production of Dietary Iron Overload: A Fundamental Cause of Disease

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Received September 1, 1994

This paper presents a review of the significant body of literature linking dietary iron overload, not only to heart disease, but also to cancer, diabetes, osteoporosis, arthritis, and possibly other disorders. Following an analysis of our understanding of the mechanistic role iron plays in oxidative damage, an interpretation of the fact that plasma concentrations of several antioxidants are decreased in the presence of disease is offered. Evaluation of (1) age-related dietary trends over time and (2) factors involved in iron absorption leads to the hypothesis that the combination of citric acid and ascorbic acid (a synergistic pair of strong enhancers) is instrumental in causing a deleterious increase in iron load in aging populations. Iron overload may be the most important common etiologic factor in the development of the diseases mentioned; therefore, the synergistic combination of citric and ascorbic acids may play a major role in our worsening disease statistics. Evidence to support this hypothesis and possible experiments to test it are included. This combination needs further study, particularly because the iron overload produced may be correctable.

Evidence for the influence of body iron load on disease has been mounting, particularly as the important role of oxidative stress in the induction of disease has come to the forefront. Considerable effort is currently being expended to understand iron's relevance to the story.

There is evidence in the literature to support the contention that dietary iron overload may be linked to heart disease, cancer, osteoporosis, arthritis, and diabetes. These diseases have been increasing in frequency in our society. As the case for iron in disease becomes better understood, we need to investigate the routes by which more people may be acquiring higher levels of iron.

There is a good possibility that chronic iron toxicity may be the single most important common factor in the development of these diseases. The influence of certain foods on iron absorption then becomes critical to our understanding of disease mechanisms. Such an understanding also offers hope, because iron levels may be amenable to correction.

This paper discusses the role of iron in disease and proposes a route by which we may be unwittingly increasing our iron stores.

HIGH IRON IN DISEASE

The genetic disorder idiopathic hemochromatosis is characterized by very high iron absorption in the presence of markedly high iron stores as measured by serum ferritin levels (1). More than 50% of patients present with diabetes (2). Cirrhosis of the liver is a common pathology in this population. Arthritis is frequently a presenting feature as well (2a). Heart disease and carcinoma (particularly hepatoma) are important late complications of this disorder (3). Between 5 and 10% of the U.S. population is postulated to be heterozygous for idiopathic hemochromatosis (1). Heterozygotes will develop relat-

1077-3150/95 $6.00

ively high body iron stores if regularly given a diet high in absorbable iron.

Patients with thalassemia, a disease of defective hemoglobin formation which requires multiple transfusions, die at a young age due to heart disease, infection, liver disease, or cancer (3). Diabetes mellitus is frequently seen in older patients with thalassemia major (4). That iron overload is responsible for this poor outcome is shown by the fact that treatment with the iron chelator desferrioxamine considerably increases life expectancy (5).

Significant iron overload has also been noted in populations whose diet exposes them to high levels of absorbable iron. Blacks of South Africa are the most notable example (6). A traditional drink is fermented in iron drums. The low pH of the beverage is likely to be responsible for the high concentration of dissolved iron in the mixture. This accounts for the high hepatic storage of iron in this population, particularly among the males (6).

In addition to the dietary iron overload seen in black subjects, a secondary ascorbic acid depletion is noted (6), caused by ferric oxidation of ascorbic acid. Low ascorbic acid, as well as reduced levels of the antioxidants α-tocopherol and retinol (7), is also found in idiopathic hemochromatosis (8).

These blacks exhibit other clinical manifestations as well. In one necropsy study, 20% of the subjects had been diabetic before death (9). These individuals were commonly underweight, middle-aged males. Spinal osteoporosis is also common among this population with dietary iron overload. The degree of osteoporosis correlates with the extent of iron overload (6). Osteoporosis has also been noted in patients with idiopathic hemochromatosis (10). Whether the osteoporosis is directly due to the iron overload or the secondary reduction of ascorbic acid is unknown. There is, however, some literature which provides a tantalizing look at a direct involvement of iron in bone resorption (11).

Thus several diseases can be linked to iron overload through these populations: heart disease, cancer, diabetes, arthritis, and osteoporosis. There may be others.

LINKS TO HEART DISEASE

In 1981 Sullivan (12) first proposed that the difference in heart disease risk between pre- and postmenopausal women was due not to a decrease in estrogen, but to the fact that postmenopausal women do not lose iron at as high a rate as when they are younger. Serum ferritin levels increase in women following menopause, although they lag behind the levels of most males (13).

Salonen *et al.* (14), in a study of Finnish men, found a correlation between high serum ferritin in the presence of high serum cholesterol and the risk of myocardial infarction. Low ferritin in the presence of high cholesterol was not associated with increased risk.

There have been several controlled studies in laboratory animals which have shown a link between iron load and the extent of reperfusion damage following ischemia (15). More specifically, higher intracellular iron, especially in the presence of ascorbic acid as a reducing agent, augments reperfusion injury. The addition of iron chelators to the perfusion medium protects against this damage, whereas addition of the antioxidant enzymes superoxide dismutase and catalase do not (15). The iron involved in the damage is present in the cell as a low-molecular-mass complex.

A recent study (16) showed that serum transferrin levels were a strong independent negative risk factor for myocardial infarction in men. Transferrin levels are normally inversely related to ferritin concentrations and thus to body iron stores (17), but in this study ferritin did not show a significant predictive ability.

It is interesting to note that the genetic model for idiopathic hemochromatosis is a homozygous hypotransferrinemic mouse (18,19). The liver, heart, and pancreas of this mouse, as in the human disorder, become heavily iron loaded. Tissue damage and iron loading patterns show similarities to the human disease, but also some differences.

It is unknown what role transferrin plays in iron absorption from the intestine, but low levels in hemochromatosis appear to contribute to uncontrolled absorption. Individuals with low transferrin levels may be heterozygous for the disorder (1,19).

LINKS TO CANCER

Iron is implicated in cancer by the high incidence of hepatoma in individuals with idiopathic hemochromatosis (20). Cholangiomas are also found (21). In addition, parenteral administration of iron, in the treatment of anemia, has caused sarcomas (22).

Between 1973 and 1989 the incidence of cancer of the liver and bile duct increased by 3% per year among black males over 65 years of age and 1.2–

1.5% per year in whites and black females in the same age group (23).

Prospective epidemiologic studies have found a link between body iron stores and cancer (24–26). These studies have found high serum ferritin and low transferrin (measures of high body iron load) to be positively correlated with the incidence of cancer.

MECHANISM OF IRON'S ROLE IN DISEASE

Iron has been linked to disease through its ability to initiate oxidative damage in a variety of situations (27). Lipid peroxidation is implicated in the mechanism of cardiovascular diseases and DNA damage leading to mutations is implicated in carcinogenesis.

In its ferrous state iron is a catalyst in the formation of hydroxyl radicals (OH·) by the Fenton reaction (28)

$$Fe^{2+} + H_2O_2 \rightarrow \text{intermediate complex} \rightarrow OH\cdot + OH^- + Fe^{3+}.$$

Such radicals have been postulated for years to be involved in numerous clinical disorders (27) to the extent that the "free radical hypothesis of disease and aging" is a major area of investigation.

Hydroxyl radicals are extremely reactive and can combine with many molecules in the cell at rates close to the diffusion-controlled limit (28). The location of the reaction in the cell is thus near the site of hydroxyl radical production. Sugars, amino acids, phospholipids, and nucleotides are all targets for this reaction. Most of the reactions of hydroxyl radicals involve hydrogen abstraction. This reaction (e.g., with a phospholipid) will form water and another free radical, which can continue the chain reaction, react with oxygen to form a peroxide radical, or combine with another radical to form a covalent compound. This is the mechanism involved in lipid peroxidation. Hydroxyl radicals can also be involved in addition reactions (e.g., with purine and pyrimidine bases) and electron transfer reactions. These reactions cause damage to cellular components and organelles, including DNA base and sugar alterations, DNA strand breaks, and impaired mitochondrial function (27).

Iron is a very active ion in initiating free radical reactions, and biologic systems have evolved efficient means to sequester it. Extracellularly, transferrin is an extremely efficient sequestrant (binding constant = 10^{22}) (29). Inside cells a small pool of iron is sequestered in ferritin (30) and as low-molecular-mass compounds, possibly complexed to citrate, ATP, or GTP (31). The location of these complexes is unclear but appears to be in vacuoles (31). Certain types of stress appear to lead to release from this location. Ischemia and mechanical damage appear to start this process, which contributes to reperfusion injury following myocardial infarction (15) and to brain injury following stroke or skull impact (30). Superoxide ion ($O_2\cdot^-$) produced by oxidant stress can release iron from intracellular ferritin (32) and thus increase free iron concentration in the cell.

Release of free iron from sequestration following cell damage may be a natural means of killing damaged tissue and, in fact, has been postulated as a form of cancer treatment: to selectively release iron within malignant cells (33). But laboratory results show tumors grow better when animals receive an iron-rich diet (34) and the release of iron in uncontrolled circumstances, such as ischemia, can lead to undesirable effects. Sublethal concentrations can reduce the cell's ability to function or cause mutations.

Anything which can cause damage to DNA may potentially cause cancer. The normal rate of oxidative damage to DNA is substantial (35). Mitochondrial DNA has a particular susceptibility. Free radicals, specifically OH· and $O_2\cdot^-$, can cause oxidative damage to DNA in the form of strand breakage and addition to purine and pyrimidine bases (27). Iron bound to DNA or present as low-molecular-mass complexes may cause damage by the production of OH·, which then quickly reacts at the site of production.

The ability of iron chelators to reduce oxidative damage to DNA (36) is consistent with a role for iron.

THE ROLE OF ASCORBIC ACID

The presence of ascorbic acid and other reducing agents (e.g., NADH) both intra- and extracellularly contributes to iron-initiated damage by catalyzing the reduction of iron(III) to iron(II) (27):

$$\text{ascorbate} + Fe^{3+} \rightarrow Fe^{2+} + \text{semidehydroascorbate radical.}$$

The iron(II) produced in this reaction can then catalyze the formation of OH· in the Fenton reaction.

The semidehydroascorbate may be removed by reaction with another molecule of itself,

2 semidehydroascorbate →
ascorbate + dehydroascorbate,

or by reduction with glutathione back to ascorbate. Dehydroascorbate may also be reduced by glutathione back to ascorbate, but this reaction is not very rapid. Dehydroascorbate is unstable and breaks down to oxalate and L-threonate, leading to a net loss of ascorbate (27). Iron(II) formed by reaction with ascorbate does not bind to transferrin for sequestration. It will be free until it can be converted to iron(III) by some means. Certain ferroxidases such as ceruloplasmin can carry out this oxidation, but these are in low concentration in some areas of the brain and other tissues (37). This allows the iron(II) to carry out free radical formation. During tissue stress more iron is released from sequestration to become involved in this sequence.

It should be noted that ascorbate plays two roles in the cell. In addition to the prooxidant reaction above, it also plays an antioxidant role (38). It can react with $O_2\cdot^-$, $HO_2\cdot$, and $OH\cdot$. to give semidehydroascorbate and an inactive molecule. In this manner it works to counteract the damage that another molecule of ascorbate may have initiated. The balance of these two competing effects, prooxidant and antioxidant, is concentration dependent (38). Prooxidant reactions predominate at low ascorbate concentrations (below 5 mM). At higher concentrations of ascorbate the antioxidant reactions predominate. The concentration of ascorbate normally present in human plasma varies from 50 to 200 μM (27). The cells with the highest ascorbate concentration (eye, spinal cord, and lung) contain 0.5–2 mM concentrations (27). Thus, concentrations normally present in tissues would be expected to be prooxidant.

Evidence for iron reduction by ascorbate *in vivo*, giving rise to the possibility of hydroxyl radical formation, is seen in the very low (often scorbutic) levels of ascorbic acid seen in patients with idiopathic hemochromatosis and dietary iron overload in the face of adequate dietary ascorbic acid (6,8). Experimental iron overload produced in the laboratory gives similar results. Supplementation of the diet of these patients with vitamin C (ascorbic acid) has been found to contribute to serious complications resulting from additional oxidative damage (39). Only a decrease in iron stores through regular phlebotomy aids in recovery of ascorbic acid levels.

It has been noted that cancer patients have low circulating ascorbic acid levels (40). This has been found in the absence of evidence of low dietary ascorbic acid (41) and supports the hypothesis that high levels of reactable low-molecular-mass iron are present in patients with certain cancers. The presence of low ascorbate levels in some cancer patients has been interpreted to indicate low dietary intake, but increased ascorbate destruction via reduction of iron(III) to iron(II) is also possible.

α-TOCOPHEROL AND RETINOL

Low plasma levels of other compounds known to act as antioxidants may also be related to high iron. Hemochromatosis patients have been found to have low levels of α-tocopherol and retinol in addition to low ascorbate levels (7). The specific prooxidant mechanism for the decrease in ascorbate is understood, but the mechanism which directly connects iron to oxidation of other antioxidants is unclear. It is possible that these antioxidants may also act as prooxidants to directly reduce iron(III) to iron(II) for use in the Fenton reaction. The recent Finnish study linking supplemental β-carotene to an increase in lung cancer among smokers (42) is consistent with such an hypothesis.

If iron is responsible for the low antioxidant levels, reduction of iron load through venesection might be more effective than increasing dietary antioxidants.

IRON METABOLISM

Dissolved intestinal iron as either Fe^{2+} or Fe^{3+} is readily absorbed by the duodenal mucosa (43). Because of the very low solubility of Fe^{3+} at duodenal pH, it must be complexed with a chelator that can cross the mucosal cell membrane. Mucosal iron is mainly bound to an iron-binding protein (binding constant 6×10^4) as the ferric ion (44). Transfer of iron from the mucosal cell to the circulation, the main point in regulation of iron absorption, is subject to a control only loosely tied to body iron stores (45,46). Transferred iron is found bound to serum transferrin (47), but the manner in which iron is released from the mucosal cell into the circulation is unclear. Iron not transferred is lost with sloughed mucosal cells. The process of absorption, therefore, consists of two steps: uptake into the mucosal cell and transfer to the circulation.

Iron is released from the transferrin by exposure to low pH. This is accomplished during a specific receptor-mediated uptake by the cells. Cell surface

receptors which bind diferric transferrin are internalized in vesicles and exposed to low pH for removal of the ferric ions, with the apotransferrin and its receptor ultimately being recycled to the cell surface. Much of the transferred dietary iron is taken up by the liver via this mechanism. As body iron load builds, the liver accumulates much of the reserve as ferritin, a protein which can store up to 4500 ferric ions per macromolecule (1).

The iron import system allows some free iron to exist in cells because transferrin never actually enters them—it is always sequestered in vesicles.

Once absorbed, iron loss is limited to desquamation of surface cells of the skin and gastrointestinal and urinary tracts and from normal minute gastrointestinal blood loss. Small amounts are lost in sweat, bile, and urine. Total daily loss in males is about 1 mg/day (3). Menstruation and pregnancy in women increase iron loss, but loss in postmenopausal women is similar to that of males (3). The high affinity of transferrin for iron contributes to this low loss rate. There is no natural mechanism for loss of excess iron.

Individuals whose excretion of iron is greater than their absorption are said to be in negative iron balance. By the same token, iron absorption greater than that lost during metabolic turnover puts one in positive iron balance. The goal in dietary iron intake would be to have sufficient iron stores to meet daily needs and to be in net zero balance (48). Because loss is limited, an extended time of positive iron balance can significantly increase iron stores.

Statistically, an iron-replete individual will transfer a smaller percentage of dietary iron to the circulation than an iron-deficient individual (49). But a replete individual on a diet high in absorbable iron is more likely to be in positive iron balance than an individual ingesting less absorbable iron. We have seen this in the iron overload of South African blacks.

Iron deficiency anemia, while probably overdiagnosed, has historically been considered a common disorder in the United States, especially among infants, premenopausal women, and vegetarians (50). More recent studies show it has declined in frequency (50,51) and the average serum ferritin (the best measure of body iron stores) levels among postmenopausal women approach those of men (13). In the face of the link between chronic iron overload and disease, it may be appropriate to shift some of our concern with iron deficiency to a concern with iron overload.

FACTORS INVOLVED IN IRON ABSORPTION

The diet contains heme and nonheme iron. Heme iron is the main form from animal sources; plants contain more nonheme iron. Each is absorbed independently through processes which are poorly understood (3). The fraction of heme iron absorbed from a normal meal is largely independent of other components of the meal and amounts to a fairly constant 25% in borderline iron-deficient individuals (52), but there is some correlation between iron stores and absorption of heme iron (46). The presence of meat (muscle tissue from any source including fish) in the diet provides some factor which aids in absorption of the heme iron (52). Meat also aids in the absorption of nonheme iron (52).

A very small proportion of nonheme iron is normally absorbed. Balanced diets generally contain sufficient iron, but the critical factor is whether the complement of other dietary components which enhance or inhibit iron absorption provides for adequate uptake (52).

Enhancers

Currently we know that meat, poultry, and fish can enhance absorption of both heme and nonheme iron by some unknown mechanism. Red meat (particularly beef) has the greatest effect on iron availability because it also includes the highest amount of heme iron in the form of myoglobin. Eggs and dairy products do not contain iron absorption enhancers (52). The most well known enhancer of nonheme iron uptake is ascorbic acid (vitamin C) (52), which acts to increase the solubility of iron by converting it to iron(II) (52). Certain organic acids, including malic, fumaric, lactic (53), and citric (54) acids, have been shown to increase the amount of absorbable iron as well. EDTA also enhances iron availability (55). The two foods known to cause the greatest enhancement of nonheme iron absorption are orange juice and sauerkraut (52). Each of these items can increase nonheme iron absorption from a mixed meal two- to fourfold (52). In the case of orange juice the components responsible are citric and ascorbic acids (54); in the case of sauerkraut, lactic acid may be the main enhancer (56), but there is a little ascorbic acid present (57). (Citric acid is high early in the fermentation process (56).)

Components which can form low-molecular-weight soluble complexes with iron, such as citric

acid, amino acids, and sugar, tend to enhance iron absorption from the gut (3).

Stomach acidity also influences iron absorption. Iron can be solubilized in the acid of the stomach, allowing it to combine in absorbable forms (3).

Inhibitors

Components known to inhibit iron absorption include carbonates, phosphates, oxalates, tannates, and phytates. These form insoluble compounds or nonabsorbable chelates with iron. Whole grains, legumes, and nuts contain high enough levels of inhibitors, especially phytate, to permit only 0–6% of iron present to be in absorbable forms (58). Fruits and vegetables contain varying amounts of these components, as well as enhancers, so the percentage of absorbable iron in these foods ranges from very low (spinach, 2%) (52) to moderate (carrots, 9%), to high (tomatoes, 21%; orange juice, 24%) (58). Milk contains the inhibitor calcium, which is thought to be the reason for the 60–80% inhibition of iron absorption in the presence of dairy products. Eggs are also inhibitors (52).

It has been found that iron bioavailability is a complicated summation of the enhancing and inhibitory effects of numerous components in the diet (52). Timing is also important. Depending on the complement of inhibitors and enhancers, absorption can vary more than fourfold from one meal (52). Although there appear to be control mechanisms which decrease the proportion of nonheme iron absorbed by iron replete individuals, a large amount of absorbable iron in the diet can still lead to iron overload, such as the previously mentioned overload seen in South African blacks (3).

Grains, legumes, and nuts were probably more important in the diets of early humans than they are today, leading to iron deficiency being a more important health factor than it is today.

While we have achieved some understanding of certain major factors involved in iron absorption, our ability to predict what the iron status of an individual might be, based on diet, is minimal, especially at the low end of the scale (50). Efforts to find a link between dietary iron levels and low hemoglobin concentrations have failed (50).

The lack of correlation between dietary iron and low iron status reflects the high variability in iron absorption due to other components in the diet. One 1971–1974 study (50) attempted to take into account meat, vitamin C, and tea intake in assessing iron bioavailability and still found no correlation between presumed bioavailability and low hemoglobin concentration or transferrin saturation (measures the researchers were using to assess anemia). Males with low hemoglobin levels had mean iron intakes similar to those of males with adequate hemoglobin levels. The same was true of females. The authors did not look at serum ferritin (50), the best measure for total body iron stores (1).

Obviously the actual bioavailability of dietary iron depends on some important absorption factor which may be known, but which has hitherto not been included in the calculations.

CITRIC ACID IN IRON ABSORPTION

Orange juice is known to increase iron uptake markedly (52,54). Reports vary as to whether the important ingredient is ascorbic or citric acid (54,58,59). When the orange juice is given simply with dissolved nonheme iron, it appears that ascorbic acid can account for the full increase in iron absorption (54). On the other hand, when iron is given in the presence of calcium (an uptake inhibitor) citric acid appears to be the more important component (54). Citric acid is also the more important orange juice component in the presence of phytate (58). The effect of the two components, ascorbic and citric acids, is more than additive, even synergistic, in the presence of absorption inhibitors both in humans (54) and *in vitro* (58). An experiment to find what the iron was complexed to in the presence of calcium and orange juice ingredients found only ferric citrate (54).

Investigation of the relative roles of these two acids in iron uptake from standard diets would appear to be a valuable avenue of research. Regardless of the component involved, orange juice addition to a meal causes very high nonheme iron absorption by greatly increasing the concentration of absorbable iron in the meal.

The binding constant for ferric citrate complex is 3×10^{12}, whereas that of ferrous citrate is 10^3 (60), but that for ferrous ascorbate is only 20 (61). The role of ascorbic acid in the reduction of iron(III) to iron(II), and not its complexing ability, seems to be the important factor in its stimulation of iron absorption (61).

HISTORIC DIETARY TRENDS IN THE UNITED STATES

It is interesting to note how the U.S. diet has changed over time with respect to enhancers and inhibitors of iron uptake (62,63). The presence of beef in our diets has decreased somewhat since 1965, after increasing slightly between 1955 and 1965 (62); milk and milk products and egg intakes have decreased (although fluid milk intake has increased slightly in postmenopausal women). Intake of grain products is down. Vegetable intake has stayed about the same, but the percent of individuals with regular intake of citrus fruits and juices, (mainly as orange juice) has increased dramatically, especially among postmenopausal women (62,63). There was a several-fold increase nationwide between 1955 and 1965 and the increase continues.

We have changed our diets to decrease some important inhibitors of absorption (milk, eggs, grains). We have decreased one enhancer (red meat), but increased another (orange juice) much more than enough to make up for the loss.

In addition to orange juice, there are now a number of popular fruit-ade products on the market which contain the citric acid/ascorbic acid combination so influential in iron absorption enhancement. A check of ingredients labels shows many non-cola carbonated beverages now use citric acid as the acidulent, and vitamin C is added to many beverages, such as soft drinks and fruit drinks (e.g., cranberry juice), which normally contain little of the compound. Citric acid and vitamin C are also added as a combination to many prepared foods.

IRON BALANCE AND AGE

At two opposing ends of the spectrum lie anemia and iron overload. The groups which are most prone to anemia are the very young (0–3 years), premenopausal and pregnant women, and those on limited diets such as vegetarians, alcoholics, and the elderly.

Over time the incidence of anemia has decreased in this country (50,51). This may partly be due to what we define as anemia (50), but is more likely due to the increased exposure of individuals to adequate and, for some, superadequate absorbable iron in their diets (50).

The groups most prone to elevated iron stores are heterozygotes and homozygotes for idiopathic hemochromatosis, adult males, and postmenopausal women (48,50). The body iron stores of the latter two groups are documented by their increasing serum ferritin levels with age (13).

Diseases that can be associated with high iron stores include diseases associated with advancing age.

AGE-RELATED DIETARY TRENDS—ASSOCIATION WITH IRON ABSORPTION

Individual dietary habits change with age (62,63). For example, small children, in general, drink lots of milk; this decreases during the teen and adult years and increases slightly at advanced age. Daily intake of soft drinks is greatest during the teen years and begins to fall off in the twenties and continues to decrease with advancing age. The subpopulation drinking soft drinks acidified with phosphoric acid versus citric acid is unknown (62,63) and would be of considerable interest since phosphate is an inhibitor and citric acid an enhancer of iron uptake (52).

Average citrus fruit and juice intake is fairly constant with age in both genders, except that postmenopausal women, 51–74, increase their intake (63). (This is mainly due to an increase in the percentage of regular users.) Average intake is decreased in the oldest group of women, 74 and over, partially due to a decrease in the percentage of individuals with regular intake (63). It is interesting to ask whether average intake and percentage of regular users in the oldest group of women decrease because women stop drinking orange juice or because a higher proportion of those women have died.

Intake of grain products is highest in the teens and decreases slightly during adulthood. Average daily intake of green and yellow vegetables is fairly constant with age in both genders. Males are the largest consumers of meat, poultry, and fish (mainly as beef, although the emphasis has decreased recently). Intake is slightly higher in middle age than at other times (63).

The age- and gender-related intake of processed food is unknown because dietary questionnaires do not generally include such specifics (64).

Any discussion of dietary trends with age and their association with iron absorption must include the likely factors which make the greatest contribution. In youth and middle age maintenance of

sufficient iron stores is the dominant objective, particularly for women. Meat, poultry, fish, and fruit juice intake, as well as decreasing dairy and grain product intake, work toward iron absorption in this age category. Soft drink and processed food intakes play an unknown role.

During advancing age, when risks of dietary iron overload increase, one particular dietary habit comes to the forefront: citrus fruit and juice intake, particularly in the most ingested form, orange juice (63).

HYPOTHESIS

For a number of years the incidence of many cancers has been increasing (23,65). Considerable effort has been expended in an attempt to identify an environmental factor that may be contributing to an increase in cancer incidence which cannot be accounted for by the aging of our population or by more effective diagnoses (66). Osteoporosis and diabetes continue to increase in incidence without adequate explanation (67–70). Heart disease continues to be the number one cause of death in the United States (71). A common etiologic factor in these diseases is their link to increased body iron stores and/or iron induced oxidative damage. The intake of heme iron has been decreasing, but the intake of the combination of ascorbic and citric acids, which causes a very large increase in absorption of nonheme iron, has increased markedly. This combination is likely to be responsible for an increase in body iron stores, which can increase risk of disease. Our major source of intake of this combination at the critical time of advancing age is orange juice (63), but processed foods and beverages also provide significant sources of the combination together in one meal (60). Anemia has been decreasing because of increased body stores of iron (50), which in excess can lead to disease. The hypothesis is that this combination contributes to our worsening disease statistics. It is important to know the extent of its contribution, which may be major.

TESTING THE HYPOTHESIS

Because of the indirect nature of the link between citric/ascorbic acid and disease, the hypothesis needs to be investigated by several different avenues.

First, more extensive research needs to be done on what effect different foods have on iron uptake and distribution in the body under realistic human dietary conditions. It would need to be shown that the combination of citric and ascorbic acids over time increases body iron levels, especially in men and postmenopausal women. (Studies to this point have concentrated on avoidance of anemia, not overload.) Since the iron and the enhancer or inhibitor must be present in the gut together, we need to look at what nonheme iron sources are being consumed along with a citric/ascorbic acid combination. In a more general sense, more extensive research needs to be done on what other combinations of foods may inhibit or enhance iron uptake under realistic human dietary conditions.

Second, we need to continue the intriguing research begun which looks at the relationship of different forms of iron in the body and human disease. Studies so far have concentrated on heart disease, but osteoporosis, diabetes, and cancer are diseases for which the hypothesis should be investigated.

Third, we need to link both steps of the process: iron uptake and disease. Questionnaires for prospective epidemiologic studies need to separate juice and fruit drink intake in terms of citric acid content as well as ascorbate content. Processed food use, especially with regard to the citric/ascorbic acid combination, should be included. Data on this combination need to be separated from the broader category of "fruits and vegetables."

A cautionary note must be added. Because it is difficult, if not impossible, to do a double blind study of this hypothesis, it is important to ensure that confounding variables are adequately controlled. For example, individuals with high intakes of vitamin C, either through diet or supplement, are less likely to be smokers, more likely to exercise regularly and have higher education levels than individuals with low intake (71a). Inadequate accounting for other variables may mask the real effect.

PRELIMINARY EVIDENCE

Considering the value put on citrus fruit as a disease preventative, an amazingly low number of epidemiologic studies have investigated its effect on disease incidence and those have mainly been limited to stomach cancer. One bit of preliminary evidence, although retrospective, comes from a recent study investigating a connection between diet and lung cancer in nonsmoking women (72). The authors separated citrus intake from that of vegetables and

other fruits. Women in the highest quintile for citrus fruit and juice intake were found to be at twice the risk ($P = 0.03$) of women in the lowest quintile for likelihood of developing lung cancer. There was an increasing trend throughout the quintiles. The authors suggest the link may be due to the possibility that women with lung cancer were treating their respiratory ailment with vitamin C and orange juice, but there was no such link with vitamin C intake alone.

There is also an intriguing bit of evidence related to the rate of increase in cancer mortality in the counties of south Florida most heavily engaged in the citrus industry (73,74). This area shows a rate of increase in mortality significantly greater than the national average during the time period of 1950–1980 for several types of cancer.

SUMMARY

Despite our efforts, the incidence of nearly every form of cancer in this country continues to rise. The notable exception is stomach cancer, which has decreased presumably due to better food storage (75) and ascorbic acid reduction of nitrosamines in the gut (76).

Considering the degree to which we now regulate exposure to synthetic carcinogens, it is unlikely that we as a nation are being environmentally exposed unwittingly to a toxic compound in sufficient concentration to account for the increased cancer incidence. Considering human propensities, it would not be surprising if the exposure is something we are doing purposely, but unknowingly, to ourselves. Recent research is highlighting the inadequacies of our current understanding of the factors in food which influence health (42,77). We need to look in new directions for connections. The combination of citric and ascorbic acids offers such a connection.

The increase in orange juice intake began in the late forties, due to increased ability to preserve and transport it as well as the increase in advertising of its possible health benefits. Its popularity has increased despite very little direct evidence of health benefits. The public is making an effort to change its diet to be more healthful, based on factors that are little understood. It behooves us to better understand the consequences of dietary changes before provoking large numbers of people to change their diets.

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