Produce for Better Health Foundation

*Fruits, Vegetables, and Health:*

# A Scientific Overview, 2011

# CONTENTS




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*Fruits, Vegetables, and Health:*

# A Scientific Overview, 2011

## INTRODUCTION

The possibility that fruit and vegetable consumption may protect human health is an intriguing prospect and has been studied around the world. Over the past few decades, the number of studies suggesting an association between fruit and vegetable intake and reduced risk of major chronic diseases has continued to grow. These studies have demonstrated that several nutrients and other components in fruits and vegetables are associated with beneficial outcomes related to disease. There is also a growing body of basic research suggesting that fruit and vegetable intake may reduce oxidation, inflammation, cell proliferation, and other important disease-related processes.

The majority of scientific investigations in humans are observational including cross-sectional, case-control, and prospective cohort studies, while relatively few are based on randomized controlled trials. (More information on these study types can be found at the conclusion of this review.) The purpose of this review is to summarize current studies related to consumption of fruits and vegetables and health. Data for the review were collected from database searches of PubMed and Medline for peer-reviewed articles published between July 1, 2006, and January 5, 2011. Key search terms included full and truncated forms of the words fruit(s), vegetable(s), fruits and vegetables,

**Over the past few decades, the number of studies suggesting an association between fruit and vegetable intake and reduced risk of major chronic diseases has continued to grow.**

### HOW FRUITS AND VEGETABLES IMPACT DISEASE RISK

New experimental studies have increasingly emerged demonstrating multiple effects of fruits and vegetables and their phytochemicals on disease-related molecular processes, signaling pathways, and cellular markers. For example, mechanisms by which dietary phytochemicals may reduce risk of cardiovascular disease include:

- antioxidant activity (i.e., scavenging free radicals, reducing oxidative stress, reducing LDL oxidation)
- induction of expression of hepatic LDL receptors
- modulation of cholesterol synthesis
- regulation of blood pressure, lipid profiles, prostanoid synthesis, and nitric oxide production
- inhibition of cholesterol absorption
- reduction of platelet aggregation and
- lowering serum C-reactive protein and other inflammatory markers

Furthermore, mechanisms by which dietary phytochemicals may reduce risk of cancer include:

- scavenging free radicals
- regulation of gene expression in cell proliferation and apoptosis
- modulation of detoxification enzymes
- stimulation of the immune system
- regulation of hormone metabolism and
- antibacterial and antiviral effects

A comprehensive and critical analysis of mechanistic experimental studies was beyond the scope of this review, but the evidence from mechanistic studies suggests that fruits and vegetables may have an even greater role to play in human health than the already positive results from observational studies outlined in this report.

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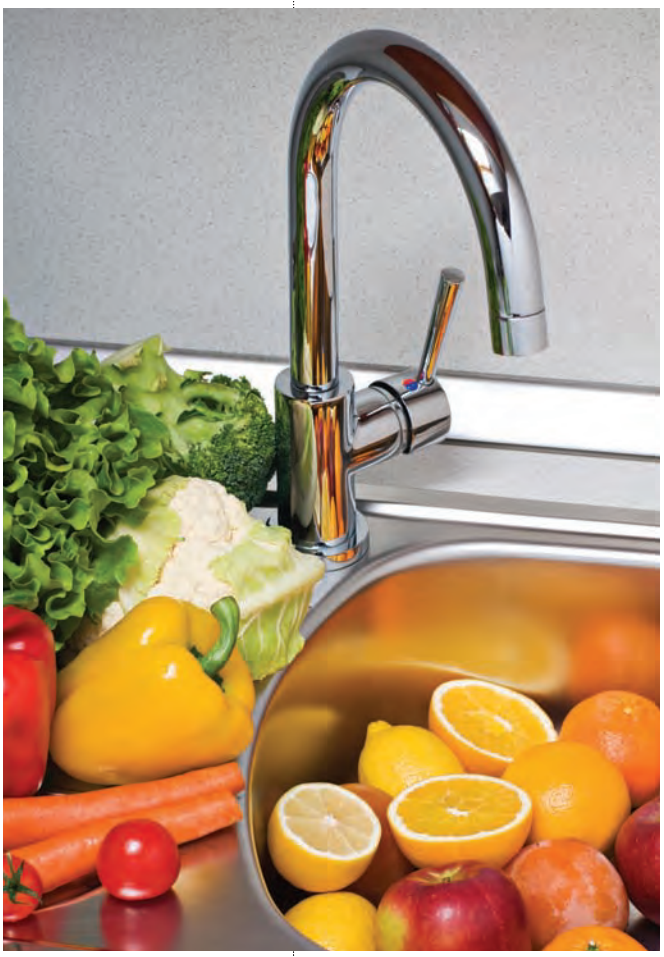
and (in alphabetical order) age, aging, Alzheimer's, arthritis, asthma, bone, birth defects, body weight, brain, cardiovascular disease, cataracts, chronic obstructive pulmonary disease, cognitive, dermatological, diabetes, diverticulosis, eye, gastrointestinal, hypertension, inflammation, life span, longevity, neurodegenerative, obesity, oxidation, skin, weight. Related articles linked to the retrieval lists were autoprompted during the search and reviewed for relevance. References cited in retrieved articles were also searched.



The current report is focused on investigations in which the effect of *total fruit and vegetable* (FV) intake was analyzed as an independent exposure and does not include studies that intended to examine individual fruits, nutrients, or components. Studies that focused solely on biomarkers of FV intake such as fiber, potassium, vitamin C, carotenoids, folate, magnesium, and other individual components are not included. However, in studies of total FV intake in which a particular nutrient or classification of fruit or vegetable was found to be protective, the findings are reported within the context of the presented study. Many researchers have examined healthy eating patterns that include FV. While this work provides important information for guiding public policy and developing guidelines, it does not advance scientific understanding of the independent effect of FV and will not be reviewed in this report.

Several investigators report the limitations of their studies and address the challenges of studying the effects of FV on disease prevention and health promotion. There is a high degree of heterogeneity among published studies, including varied approaches to classifying, grouping, and/or excluding FV from an analysis, divergent designations of FV serving size, multiple methods of assessing dietary intake, inconsistent adjustment for confounders, and different outcome measures, to name a few. Several systematic reviews and meta-analyses have attempted to address these issues and provide quantifiable evidence for an effect of FV intake on risk of important major diseases. A brief overview of these reviews is provided, although the current report is focused on individual studies.

An ongoing methodological issue is the observation that individuals with higher intakes of FV are likely to have health-related behavioral and social characteristics that differ from those who are low consumers, including higher education and socioeconomic status, lower BMI, and a greater tendency to engage in physical activity, take vitamin supplements, and avoid smoking. Furthermore, the addition of FV to the diet of free living individuals often results in reduced intake or displacement of dietary fat and/or sodium, each of which is likely to result in favorable health-related outcomes. Although most analyses provide statistical adjustment for many of these factors, it is possible that unknown, residual confounding effects associated with FV intake are not accounted for.

There are many plausible mechanisms by which FV intake might be protective. A body of increasingly sophisticated research has emerged demonstrating multiple effects of FV and components on disease-related molecular processes, signaling pathways, and cellular markers. A comprehensive and critical analysis of this work is beyond the scope of the current review. Discussion of mechanisms will be limited to individual components within the context of studies that examine FV in their intact form.

The current report is organized in alphabetical order by disease, condition, or process, including a summary of relevant studies and a brief conclusion for each, followed by an overall summary and evaluation of evidence.

## ARTHRITIS

Fruit intake may have a beneficial effect on pathogenic indicators that predict future osteoarthritis in the knee.[1] An Australian study published in 2007 evaluated baseline dietary data from a 121-item food frequency questionnaire (FFQ) to determine quartiles of FV intake of 297 subjects (age (58 ± 5.5 years) in the Melbourne Collaborative Cohort Study. Ten years after baseline, each subject had an MRI for assessment of several measures, including tibial cartilage area and bone size, cartilage defects, and bone marrow lesions. After adjusting for confounders such as body weight, fruit intake was inversely associated with bone measures including tibial bone area (P=0.04) and a 28% reduction in bone marrow lesions (P=0.05). Vitamin C intake was also correlated with these markers, but vegetable intake was not. This study suggests that fruit, as a source of vitamin C, but possibly by other mechanisms, may have a role in preventing bony changes that occur early in the development of osteoarthritis in the knee. A limitation of this study is the 10-year span between baseline diet assessment and collection of MRI data. The findings of the current study remain to be confirmed with long-term prospective trials.

Given the inflammatory processes associated with arthritis, it is possible that FV intake might be beneficial (see section on Inflammation). Earlier published data hinted at a link between components in FV and processes associated with rheumatoid arthritis, although no subsequent work has been published in this area.

**This study suggests that fruit, as a source of vitamin C, but possibly by other mechanisms, may have a role in preventing bony changes that occur early in the development of osteoarthritis in the knee.**

## BONE HEALTH

Bone health is related to a combination of genetic factors, lifestyle habits, and nutrition. Fruits and vegetables contain a number of components that are potentially beneficial to bone health and may attenuate loss of bone mass. The majority of recent studies have focused on bone mineral density and markers of bone metabolism. Others have assessed risk and/or fractures in postmenopausal women due to the high risk of this population for increased bone fragility and deterioration in the architecture of bone (osteoporosis).

A concise systematic review of 8 studies of FV and bone health was published in 2010.[2] It was reported that the majority of work in this area is observational, including case-control, cross-sectional, and prospective studies, with only two randomized controlled trials published in the past few years. The collective results of the studies were mixed. After controlling for dietary and risk factors related to bone health, only three cross-sectional studies demonstrated a positive effect of FV intake on bone mineral density, although not consistently at all bone sites (lumbar spine, forearm, femoral neck, or total hip).

Markers of bone turnover were not associated with FV intake in a randomized controlled clinical trial of 276 post-menopausal women after 2 years of intervention.[3] This trial is of particular interest because 1 arm of the study included the addition of FV to the diet each day (recommended +300 grams but actual intake ranged from 126-1470 g/day for fruit and 31-630 g/day for vegetables). Plasma levels of vitamin C were increased in this group (P=0.05) after one year but were not different after the second year. The other 3 arms of this study included potassium citrate supplementation at high and low doses and a placebo control. Bone mineral density in the spine and total hip were not affected by any of the treatments. The wide variability in reported consumption of FV in this study suggests the challenge of controlling quantities of FV intake in free living subjects and maintaining dietary compliance over an extended period of time.

One cross-sectional study reported a significant association between FV intake and bone mineral density in 670 post-menopausal Chinese women, average age 55 ± 3.5 years.[4] Subjects were stratified into quintiles of total FV intake based on dietary assessments conducted by trained interviewers and a 60-item FFQ. There was a significant and independent positive association between total FV intake and bone mineral density at all measured sites, including whole body, lumbar spine, left total hip, trochanter, and intertrochanter. A significant linear trend between total FV intake and bone mineral density of the whole body (P=0.024) and lumbar spine (P=0.003) was also reported.

Further well-controlled, long-term trials with standardized techniques need to be conducted in order to conclusively state that fruit and vegetable intake can improve bone health or reduce risk of osteoporosis.

A recent study in Korea evaluated the diets of 524 healthy men and women, ages 20-29, using 24-hour recalls and trained interviewers.[5] The average intake of FV was >300 grams in both genders across three age groups of 20-29, 30-49, and 50-64 years. The investigators provided a detailed report of estimated daily nutrient intakes and the most common 20 FV consumed by each age group. An analysis of serum calcium, osteocalcin, and deoxypyridinoline (DPD) excretion in a small subset of younger subjects (n=51, age 20-29 years) did not show an association with FV intake, except for a positive association with tuber vegetables. Several limitations to this study, including the restricted subset analysis, suggest that further work needs to be completed to accurately determine the relevance of FV on bone health in Korea.

The authors of the aforementioned systematic review noted a high risk of bias in at least 4 of the 8 available studies of FV intake and bone health. The mixed findings of the current research indicate that further well-controlled, long-term trials with standardized techniques need to be conducted in order to conclusively state that FV intake can improve bone health or reduce risk of osteoporosis.

## CANCER

A new review of cancer and FV provided a brief historical perspective dating back to 1975, at which time the first observation between FV intake and reduced risk of cancer was reported.[6] Subsequent to the early findings, hundreds of studies were published, particularly case-control reports, that ultimately led to the consensus that there was "convincing" evidence that high intakes of FV could decrease the risk for cancer, including cancers of the mouth and pharynx, esophagus, stomach, colorectum, and lung. However, recent and larger studies have not confirmed the earlier findings. As such, in 2007 the World Cancer Research Fund/American Institute for Cancer Research downgraded previous conclusions and reported that a link between FV intake and cancer might be "probable" for some cancers or "limited suggestive" for others.[7] However, cancer is a complex process and remains an active area of investigation with many ongoing studies still attempting to define the true nature of the association between FV intake and cancer.

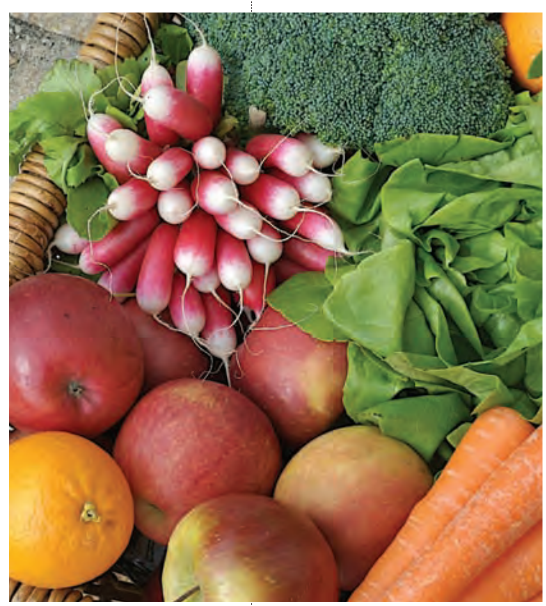
### *Overall Cancer Risk*

Several studies have evaluated the association between FV intake and risk of total cancer incidence in prospective cohort studies, with two of the most recent also being the largest trials. A recent analysis of the European Prospective Investigation into Cancer and Nutrition (EPIC), including data from 10 European countries, was published in 2009. In this study, 478,478 men and women were followed for a median of 8.7 years, and 30,604 cases of cancer were reported. There was a very small inverse association between FV intake and cancer, with a 3% reduced risk for every 200 g increase in FV intake. Stratified analysis showed that this reduction was mainly attributed to cancers associated with smoking and alcohol consumption, and the benefit was stronger in subjects with high alcohol consumption.

The National Institutes of Health-American Association of Retired Persons (NIH-AARP) Diet and Health Study in the U.S.A. included members from AARP, aged 50-71 years, from 6 states and metropolitan areas. A recent analysis of this study included 483,338 subjects (195,229 women and 288,109 men) and 50,863 incident cancer cases over a mean follow-up period of 6.9 years.[8] Based on data from a self-administered, 124-item validated FFQ, quintiles of FV intake (cup equivalents per 1000 kcal) were determined and used to assess the relative risk of cancer. The data did not support an association between FV intake and total cancer in this cohort, after correction for potential confounders, including smoking in men and menopausal hormone therapy in women.

A smaller prospective study followed a group of 44,838 Swedish women enrolled in the Swedish Women's Lifestyle and Health Study for an average of 14 years (aged 30-49 years at recruitment).[9] There were 2347 incident cases of cancer diagnosed at an average age of 53 years. FV intake was assessed with a FFQ (80 item), although notably, total fruit intake was analyzed as the sum of just 3 fruits (apple, banana, and orange) and fruit juice. Vegetable intake was the sum of 10 different vegetables. There were no statistically significant associations found between FV intake and risk of cancer. Although these findings align with the outcomes of the aforementioned larger trials, this was a much smaller study with a younger population than other cohorts, and 46% of the cancer cases were breast cancer. The limited variety of FV and, as the authors speculated, the relatively low median intake of FV in this group (204 g/day) might also have accounted for the null effects. Another relatively small prospective analysis (n=25,623, 851 cases) of the Greek cohort of the EPIC study reported a much higher intake of FV (median intake of 837 g/day) and observed a reduced risk of overall cancer over a median 7.9 years of follow-up.[10]

Recent case-control investigations have shown mixed results regarding a potential association between FV and total cancer risk. In a study conducted in Uruguay, including 3539 cancer cases and 2032 controls, an inverse association between high FV intake and total cancer was observed in men.[11] However, stratification showed that the beneficial effects were limited to male smokers with higher meat and alcohol intake, suggesting possible confounding effects of smoking and alcohol as observed in the large prospective trials previously described.



Several summaries and reviews have provided a composite assessment of the effects of FV intake on various common cancers. A recent review examining the effects of components in the Mediterranean diet included a summary of FV and the occurrence of cancer.[12] The report was based on "an integrated series of multi-centric case-control studies conducted in Italy," including data from approximately 10,000 subjects and 17,000 controls studied between 1991 and 1997. The odds ratios showed reduced risk associated with vegetable intake, particularly for cancers of the digestive tract, ranging from a 20-70% lower risk between the highest and lowest levels of intake. Prostate cancer was not affected. A weaker effect was reported for fruit intake (10-30% reduction), although several cancers were unaffected by fruit intake.

A newly published review included a summary from several large prospective trials that examined the risk of common types of cancer.[6] Each will be briefly discussed in this overview, with additional data from recent trials that have examined the link between FV intake and cancer at several sites.

### *Breast Cancer*

A recent review addressing work done between 2003-2007 suggested high intakes of FV are unlikely to have a significant protective effect on the risk of breast cancer. However, it was noted that specific vegetables, particularly if rich in isoflavones (e.g. soy beans), might be effective in reducing estrogen stimulation and thus be protective against some forms of breast cancer. Three reports published in 2009-2010 identified reduced risk of breast cancer in association with vegetable intake.

The Black Women's Health Study, a prospective cohort of 51,928 American women (ages 21-69 years) followed for 12 years, identified 1268 incident cases of breast cancer.[13] Based on FFQ assessment it was found that total vegetable intake (but not total FV intake) was associated with a 43% reduction in risk of estrogen and progesterone-receptor negative breast cancer in women consuming more than 2 FV servings/day, compared to those eating less than 4/week. Cruciferous vegetables and carrots were identified as protective for all breast cancers.

A case-control study in China (equal cases vs. matched controls, n=438) also identified cruciferous vegetables and carrots as potentially protective against breast cancer.[14] In addition, total vegetable intake was associated with a 72% reduced risk, and total fruit, a 47% reduction. Several other individual FV, including green leafy vegetables, tomatoes, and bananas, were associated with lower risk.

A case-control study in Korea (n=358 and 360, respectively) showed that total vegetable intake was associated with reduced risk of breast cancer, although pickled vegetables actually increased risk.[15] However, an earlier case-control study in Korea from a different group found no effect of FV on breast cancer risk, although tomatoes, grapes, and cooked soy beans were identified as protective.[16]

### *Prostate Cancer*

FV intake was not associated with reduced risk of prostate cancer in a recent prospective study of 29,361 men (1338 cases over 4.2 years).[17] Vegetable intake, particularly cruciferous, was associated with reduced risk of aggressive, extra-prostatic prostate cancer (stage III or IV tumors). In general, there have been inconsistent results regarding the link between FV intake and prostate cancer, so the association is inconclusive.[6]

**There are many benefits to consuming fruits and vegetables and multiple, plausible mechanisms by which fruits and vegetables might be protective against cancer, such as reducing oxidative damage, detoxifying carcinogens, regulating cell proliferation, modifying cell signaling, and others.**

### *Colon and Colorectal Cancer*

A pooled analysis of colon (not colorectal) cancer risk in 14 prospective studies published prior to 2005 was completed and reported in 2007.[18] The analysis suggested that FV intake is not strongly correlated with colon cancer risk overall but may be associated with lower risk of distal colon cancer. Subsequent work on colorectal cancer has not demonstrated a protective role for FV in reducing risk.

A recent study investigated the potential of FV to reduce colorectal adenoma, a precursor of colorectal cancer in men and women who were screened for colorectal cancer in the multisite Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO) in the U.S.[19] Fruit intake (highest versus lowest quintile intake) but not vegetable was associated with reduced risk of colon but not rectal adenoma. The observed reduction with fruit was 75% ($P<0.001$), with intake of 5.7 fruit servings/day (Pyramid servings) versus 1.2 servings/day.

### *Lung Cancer*

Studies of lung cancer have shown that patients with lung cancer have a lower intake of FV than healthy controls, but conclusive associations are complicated by the strong confounding effect of smoking. Cruciferous vegetables may be modestly but inversely associated with lung cancer risk.[20] In a new analysis of the EPIC cohort (n=452,187, followed for 8.7 years), an inverse association was reported between lung cancer and increased variety of vegetable consumption, mainly in current smokers.[21] For squamous cell carcinomas, greater variety in total FV (combined and separately) reduced risk in smokers. This finding is from one of a few new studies suggesting that variety might be an important factor in the protective effect of FV in human health.

### *Esophageal Cancer*

Reduced risk of esophageal cancer has been reported in a number of case-control studies, with risk reduction of up to 40-50%.[6] A recent case-control report examined the diets of patients with Barrett's esophagus, a potential precursor to esophageal carcinoma, and found that FV intake was strongly associated with reduced risk.[22] However, prospective trials have been inconsistent.

### *Gastric Cancer*

High intake of fresh FV, particularly raw and allium vegetables and citrus fruits, may reduce the risk of gastric cancer. Composite reports of case-control studies and cohort studies have estimated risk reductions ranging from 15-37% for fruit and 6-34% for vegetables.[23] However, recent prospective trials have not consistently supported the finding of an inverse effect of FV intake on gastric cancer.

### *Oropharyngeal Cancer*

The majority of available data related to FV and oropharyngeal cancer are based on case-control and observational studies which suggest an approximate 50% risk reduction associated with FV intake.[6, 24] The duration of exposure and the type of FV are important, with citrus having a strong inverse association.

In conclusion, there are many benefits to consuming FV and multiple, plausible mechanisms by which FV might be protective against cancer, such as reducing oxidative damage, detoxifying carcinogens, regulating cell proliferation, modifying cell signaling, and others. There is clearly a need for well-controlled longitudinal studies to advance our understanding of the true association between dietary FV and cancer.

## CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD) includes diseases of the blood vessels supplying the heart (coronary heart disease, CHD), brain, and periphery, among other disorders. The World Health Organization reports that CVD, particularly CHD and stroke, is the leading cause of death globally, representing 29% of worldwide deaths, with 82% of these occurring in low- to middle-income countries.[25] CVD is projected to remain the top killer, with an estimated 23.6 million deaths predicted by 2030.

A large body of observational evidence from earlier population studies suggested that cohorts consuming higher quantities of FV experienced fewer CVD-related events and death and had favorable risk profiles compared to those with reduced consumption. A 2007 meta-analysis of 12 studies (13 cohorts) published between 1992 and 2004 showed that consumption of more than 5 servings of FV/day (>391 g) was associated with a 17% reduced risk of CHD (P<0.001).[26] A similar analysis published a year earlier included 6 cohort studies and showed a 4% reduction in CHD risk for each additional portion/day of FV intake.[27]

Dauchet and Dallongeville recently addressed these analyses and completed a summary of studies and a critique of evidence related to FV intake and the incidence of CHD.[28] The review focused on prospective cohort studies with endpoints including CHD events, CHD-related mortality, and CVD events. Cohort sizes ranged from a few hundred to over 100,000 subjects, with follow-up periods between 3 and 26 years. FFQs were most often used to assess dietary intake, and studies grouped total FV and/or FV intake separately. The authors of the review concluded that the risk-reduction estimates from prospective cohort studies are less impressive than initially observed in ecological or case-control studies. They cited the shifts in other health-related dietary factors in the population that have occurred over the span of the 50 years covered by the reviewed studies (such as fatty acid composition). Furthermore, the majority of the current findings are based on work conducted in North America and Europe. The authors cautioned against concluding a causal link between FV intake and reduced CHD, due to the heterogeneity of epidemiological trials and the lack of well-controlled clinical trials in this area.[28]

Since the publication of the above meta-analyses and review, there has been additional new work evaluating the association between dietary FV and risk of CVD and, importantly, some investigations have been completed in the non-Western world. A case-control study of 290 patients (67% male, age range 23-79 years) in Serbia suggested that FV intake differed between patients and matched controls and that FV was protective against a first episode of acute myocardial infarction (MI) or unstable angina.[29] The authors calculated 3 times higher odds of CHD in subjects who consumed vegetables infrequently (<1 cup/week, P<0.01) compared to more than one cup/day. For fruit and

**An updated analysis of 8 European cohorts enrolled in the ongoing European Prospective Investigation into Cancer and Nutrition (EPIC) was reported in January 2011 after completion of this review. The data included 313,074 men and women followed for an average of 8.4 years and showed that consumption of 8 portions of fruits and vegetables per day (80 g/portion) was associated with a significant 22% reduction in CHD mortality compared to <3 portions per day. This finding adds to evidence suggesting that the quantity of fruits and vegetables might be an important factor in reducing risk of CVD and other chronic diseases.**

Source: Crowe FL, Roddam AW, Key TJ, et al. Fruit and vegetable intake and mortality from ischaemic heart disease: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Heart study. 2010 *Eur Heart J*: doi:10.1093/eurheartj/ehq465.

fruit juice, an independent analysis showed a 1.78 higher risk associated with low consumption (P<0.05, <0.001 for fruit and juice, respectively). The findings of this study align with other case-control studies showing that FV intake might reduce risk of CHD, although it is noteworthy that the authors did not indicate if the questionnaire used to assess dietary intake was validated, and they did not test for interactions between BMI or smoking (both higher in cases than control) and diet.

A Dutch cohort enrolled in the Monitoring Project on Risk Factors and Chronic Diseases in the Netherlands was examined to determine the effect of raw and processed FV intake on the incidence of acute MI or fatal CHD-related events.[30] Subjects completed a FFQ including 35 FV, differentiating raw, cooked, or juices/sauces to determine quartiles of intake (excluding potatoes and legumes). Total FV intake was associated with a 34% lower incidence of CHD (P=0.04) over 10 years, in subjects consuming a median of 589 g/day in the fourth quartile of intake, compared to the lowest median intake of 292 g/day. Raw and processed FV tended to be inversely associated with MI or fatal CHD-related events (P=0.08 for raw and P=0.14 for processed).

**Total fruit and vegetable intake was associated with a 34% lower incidence of CHD over 10 years in subjects consuming a median of 589 g/day in the fourth quartile of intake, compared to the lowest median intake of 292 g/day.**

A cohort study in Denmark used FFQ data (192 items) from a large ongoing trial, the prospective Danish Diet, Cancer and Health Study, to examine the link between increased FV intake and acute coronary syndrome (ACS), including MI, unstable angina, and cardiac arrest.[31] There was a tendency for FV to reduce the risk of ACS for every 100 g/day increase in FV intake, although in models adjusted for several potential lifestyle and diet confounders, the reduction was nonsignificant. Conversely, a 4% higher risk of ACS was found in women with increased fruit juice intake. This was a large study (n=53,383 men and women, aged 50-64 years) with an average 7.7 year follow-up, although the a priori fruit groupings to investigate citrus fruits and apples may limit the general applicability of the findings.

A recent analysis of a large prospective cohort in Japan (n=77,891) demonstrated a protective effect for total fruit, but not vegetable or total FV intake, on the risk of CVD (MI and stroke).[32] Subjects completed a 138-item validated FFQ and were followed for either 7 years or 4 years (data for two cohorts combined). A significant inverse association was found for fruit intake in the highest quartile (median 733 g/day) versus the lowest quartile (186 g/day), with a 19% reduced risk (P<0.01). A stratified analysis demonstrated that the effect of fruit was influenced by gender and smoking status; nonsmokers experienced a 24% reduced risk in the highest quartile of intake (P=0.02). Women with the highest fruit intake were at 23% lower risk (P=04), and the reduced risk associated with total FV intake approached significance in women. In men, risk reduction with fruit (17%) approached statistical significance but total FV was not protective. In Japan, stroke accounts for most of the CVD, which differs from studies in Western populations where CHD is much higher. This study and others suggest that fruit is more protective than vegetables in reducing risk of stroke (see section on Stroke).

As noted in the aforementioned study, there may be important interactions between lifestyle and dietary behaviors or underlying conditions that influence the potential effect of FV consumption on cardiovascular risk. While many have been identified as potential confounders and statistical adjustments may control for their effect, it is important to understand and integrate background dietary factors. Since olive oil is prevalent in the diet of Italians, a large prospective study of 29,689 women from 5 EPIC cohorts sought to determine the association between FV intake, olive oil, and CHD.[33] An adapted FFQ with images was completed by participants (46.6% postmenopausal, average 50 ± 7.9 years), and subsequent incidence of MI, revascularization, or death was determined over nearly 8 years of follow-up. Olive oil intake averaged 24.7 ± 13 g/day and was strongly and significantly correlated with total vegetable intake, as well as green leafy vegetables and raw tomato consumption (P<0.0001 for each). There was a significant 46% reduction in CHD risk associated with green leafy vegetables (P=0.03 for trend) between the highest quartile of intake (>50.8 g/day) and the lowest quartile, ≤17.6 g/day. A risk reduction of 44% was also noted for olive oil in high versus low quartiles (>31.2 vs. ≤15.9 g/day, respectively, P=0.04).

Another study considered the interaction between dietary carbohydrate and the effect of FV intake on risk of ischemic CVD (MI, stroke, both fatal and nonfatal).[34] FFQ data from two large, ongoing American trials, including the Nurses' Health Study and the Health Professionals' Follow-Up Study, was used to stratify carbohydrate intake as low (<40% of daily kcal), medium (>40-55%) or high (>55%), as well as determine FV consumption, excluding potatoes and beans/lentils. The validated 126-item FFQ was administered for self-completion by mail every 4 years. During 16 years of follow-up for women (n=70,870) and 14 years for men (n=38,918), a modest non-significant inverse association between FV intake and CVD was observed in the groups with low carbohydrate intake but not in those with medium or high intake. Low carbohydrate intake was associated with fewer healthy habits compared to high carbohydrate consumers, including more smoking and lower physical activity. Total vegetables, green leafy vegetables, and carotene-rich FV were also associated with reduced risk and increased carbohydrate intake. The authors concluded that it is important to maintain intake of at least 5 servings of FV when dietary carbohydrate is low.

### *Stroke*

As indicated earlier, stroke is among the leading causes of CVD-related death. A widely cited meta-analysis published in 2006 provided an analysis of 8 prospective cohort studies published between 1995-2003.[35] The data covered 257,551 subjects, including 4917 stroke events, both ischemic and hemorrhagic. The pooled risk reduction associated with consumption of 3-5 servings of FV/day was 11% and rose to 26% in those consuming more than 5 FV/day, compared to individuals consuming less than 3 servings of FV/day. The authors concluded that these data strongly support recommendations to consume more than 5 servings of FV each day.

One recent study related to FV and stroke was published in 2009 and included data from the Finnish Mobile Health Examination Survey.[36] A cohort of 3932 men and women were followed for 24 years, during which time there were 675 cases of cerebrovascular disease, ischemic stroke, or intracerebral hemorrhage. Baseline diet interviews (validated on two occasions) were conducted to assess intake of FV and other food groups and determine quartiles of intake. The analysis showed a significant reduction in risk associated with the highest quartile of fruit intake compared to the lowest, with differences ranging from 25-53% in reduced odds of cerebrovascular disease, ischemic stroke, or intracerebral hemorrhage (P=0.006 to 0.03). Sub-analyses indicated this effect was mainly due to the intake of citrus fruits, including juices. Cruciferous vegetables were the only vegetable group associated with reduced risk of all three endpoints, ranging from a 21-51% reduction. Legumes and root vegetables each significantly reduced ischemic stroke risk by 28% (P=0.02 and 0.03, respectively).

### *Risk Factors and Processes Related to CVD*

Several studies have examined the effect of FV intake on well-established cardiovascular risk factors, including hypertension as well as related processes such as inflammation and oxidation.

### Hypertension

A well-designed, placebo-controlled crossover study to test the effect of raising dietary potassium (K) levels by either increased FV intake or K supplementation did not show an effect of FV intake on BP or markers of endothelial function.[37] The study was adequately powered to detect a 4 mm Hg change in BP in 48 overweight subjects of mixed origin (25 females; 23 males). Subjects with moderate- or pre-hypertension (mean BP systolic/diastolic 137/89) completed 4 intervention periods, in random order, each separated by 5-week minimum washout periods. Experimental treatments were 6 weeks each and included either high FV intake (providing 20-40 mmol K/day), or 40 mmol K/day as potassium citrate capsules. Control treatments over 6 weeks included either 15 mmol K/day from FV or placebo capsules. Compliance measures included capsule counts, food record analysis, and urinary K excretion. The results showed that ambulatory BP readings over 24 hours were not affected by increased FV or K supplementation compared to control. Furthermore, FV intake did not impact endothelial function, assessed by flow-mediated dilation studies. This study is one of the few to use a crossover design in which variability is minimized compared to other approaches. The results do not support the hypothesis that FV intake is associated with reduced BP in early hypertension. However, it is possible that moderate hypertension, the study duration, the 5-week washout periods, or other variables accounted for the lack of an observed effect in this study.

A prospective study in Spain examined the association between self-reported FV intake based on validated FFQs and self-reported blood pressure (validated for accuracy in a random subsample).[38] The study aimed to examine the effects of FV intake in subjects with a diet relatively high in olive oil, characteristic of the Mediterranean diet. A total of 8594 subjects from an ongoing cohort study of diet and disease prevention (the Seguimiento University of Navarra or SUN Project) were followed for over 49 months. A comparison of baseline quartiles of intake showed there was no significant effect of higher intake of fruit, vegetables, or combined FV on hypertension, except among participants with low olive oil consumption (<15 g/day). Baseline reported FV intake appeared to be predictive of subsequent BP in the follow-up period but was only statistically significant for fruit and diastolic blood pressure. The authors postulated that the relatively young mean age of subjects in their study (range 38.9 to 42.1) across quartiles of FV consumption might account for the lack of an association found in similar trials.

In contrast to the above, a study of longer duration, also conducted in the UK, found a significant association between FV intake and endothelium-dependent relaxation in hypertensive overweight patients, aged 52-56.[39] All subjects (n=117, 62-70% males) had a mean habitual intake of 2.3 servings FV/day prior to the study. After a 4-week washout period of 1 FV/day, each was randomized to a diet group that averaged 1.1, 3.2, or 5.6 servings FV/day (based on UK Food Standard Agency guidelines using household measures). Compliance was assessed with plasma biomarkers and a series of 4-day food records at baseline, run-in, and after 4 and 8 weeks in the study. A significant dose-response effect to acetylcholine-induced, endothelial-dependent relaxation was observed, resulting in a 6.2% increase in maximum response for each extra portion of fruit consumed. Serum levels of the carotenoids, lutein, and β-cryptoxanthin also increased in a dose-dependent manner across the groups. Although the study was not powered to determine a significant change in systolic blood pressure, the authors did note a trend toward reduced systolic BP with increasing FV intake. One limitation of this study was the inclusion of subjects on anti-hypertensive and lipid-lowering medications, and although medication doses were maintained at a constant level, it is possible these might have affected vascular reactivity.

In order to achieve a realistic assessment of blood pressure response and avoid the "white coat effect" of elevated blood pressure readings in a medical setting, a prospective investigation in Japan used home monitoring systems to examine the effect of dietary FV intake on future predicted blood pressure response. A baseline average BP of home readings (using a semi-automated monitor over a 4-week period) was determined for a subset of 745 normotensive subjects (274 men; 471 women) enrolled in a larger, ongoing community-based observational trial, the Ohasama study.[40] Baseline FV intake was assessed using a validated 141-item FFQ adapted for the Japanese diet. Follow-up BP measurements were completed 4 years after baseline to compare blood pressure response based on high and low quartiles of FV intake. At follow-up, 222 subjects (29.8%) were identified as hypertensive (BP values ≥135/85 mm Hg; n=70 on anti-hypertensive medication). After adjustment for multiple confounders, there was a 60% lower risk of hypertension in the highest quartile of baseline fruit intake compared to the lowest quartile (P=0.004) which was attenuated but still significant after adjustment for related nutrients including potassium, B-carotene, folate, vitamin C, and total fiber (55% lower risk; P=0.025). There was no association between any of the nutrients, and hypertension and vegetable intake was not found to be protective. The most common fruits consumed included, in order: citrus, apple, grape, and watermelon. Although there was no statistically significant interaction between BMI and fruit intake (P=0.10), overweight subjects (BMI >25) had much lower odds of being hypertensive (79% reduction) if they were in the highest quartile of fruit intake (P=0.005). The prospective design of this study was a strength, although the authors acknowledged a possible selection bias since only 54.1% of eligible subjects enrolled in the study, and the number of subjects was relatively small for a study of this nature.

**Collectively, there are promising data suggesting a link between fruit and vegetable intake and reduced pre and full hypertension, although there is a need for randomized controlled trials to confirm an association and determine the optimal duration of exposure and the quantity of fruits and vegetables needed.**

Newly diagnosed diabetics, with hypertension (n=220) and without (n=230), were investigated in a case-control study in Turkey to determine if several dietary variables, including FV, might be associated with hypertension in this population.[41] Each subject was interviewed and completed a 65-item adapted FFQ. Fruit and vegetable intake was negatively correlated with blood pressure in the control group but not in diabetic patients (P<0.01). The consumption of FV was also a significant, independent predictor of hypertension.

Collectively, there are promising data suggesting a link between FV intake and reduced pre- and full hypertension, although there is a need for randomized controlled trials to confirm an association and determine the optimal duration of exposure and the quantity of FV needed.

**Inflammation**

Inflammatory processes have been implicated in CVD as well as other chronic diseases. A few recent studies have investigated the effect of FV consumption on common inflammatory markers, in particular, plasma levels of C-reactive protein (CRP), an acute phase-reactant protein associated with CVD.

A cross-sectional study of 486 female teachers aged 40-60 years in Tehran showed that plasma levels of CRP were correlated with FV intake.[42] Intake of FV was determined using a validated 168-item FFQ administered by a dietitian. In crude and adjusted models there was a significant and inverse association between plasma levels of CRP and increasing quintiles of fruit, vegetables, and total FV intake (P<0.05, P< 0.01 in fully adjusted models for fruits and vegetables, respectively). Mean intake was 362 ± 6 g/day and 307 ± 9 g/day for the highest quintile of fruit and vegetable, respectively, compared to means of 98 ± 7 g/day for fruit and 142 ± 7 g/day for vegetables (excluding potatoes). The authors also reported a significant reduction in the risk of metabolic syndrome between quartiles, with a 34% reduction associated with high fruit intake, 30% for vegetables, and 39% for FV combined, although the significance of these reductions was reduced after adjustment for BMI (P<0.09 fruit; P<0.07 vegetable).

A cross-sectional study of 1060 subjects in Portugal also showed that FV intake was linked to CRP levels but only in male subjects.[43] Fruit and vegetable intake was determined with a validated, semi-

The available data related to inflammatory markers is promising and suggests a mechanism by which fruit and vegetable intake may be protective in a variety of conditions in humans.

quantitative 82-item FFQ. Plasma levels of CRP were measured and categorized in one of three groups, either: no risk, moderate risk, or high risk, to determine if increasing FV intake would be associated with the risk category. The results showed that each 100 g increment in FV intake reduced the odds of shifting to a higher risk category of CRP by 27% in normal-weight men (n=126) and 14% in overweight men (n=259). Incremental increases in fruit intake reduced risk by 27% and 20% in normal and overweight men, respectively. Intake of vitamins E, C, and fiber were also negatively associated with CRP. There were no significant correlations between FV intake and any of the nutrients with CRP in normal-weight or overweight women (total n=675).

Another cross-sectional study in an older population of 3258 men (60-79 years) living throughout Britain showed similar but weaker correlations between FV intake and inflammatory markers.[44] Fruit, but not vegetable intake, was inversely associated with plasma levels of CRP (P=0.05), tissue plasminogen activator (t-PA), antigen (P=0.02), and blood viscosity (P=0.01) in the fully adjusted models in this study.

One interesting study sought to determine if variety of FV intake, rather than absolute quantity, might be protective against CRP.[45] An adapted, validated FFQ was used to quantify FV intake of 1159 Puerto Rican adults (324 men and 835 women), ages 45-75, who were enrolled in the Boston Puerto Rican Health Study. There was a significant, 3-fold difference in the highest tertile of FV intake (5.9 servings/day) and lowest tertile (2.3/day). Those in the highest tertile also had twice the variety of FV and had higher plasma levels of ascorbic acid, folate, and carotenoids. The investigators found a significant inverse association between plasma CRP level and greater variety of FV intake, while the association between total quantity of intake and CRP did not reach statistical significance.

A recent study in adolescents added to data suggesting that FV intake might modulate inflammation.[46] Students (n=155 boys, 130 girls; 20% African American) were recruited from a public school in Minneapolis to participate in a cross-sectional study. Dietary intake was assessed at baseline (mean age = 13) and 2 years later (mean age = 15 ± 1.2 years) using a validated 127-item FFQ. Several markers of inflammation in blood and overnight urine were assayed at the 2-year mark. Average FV consumption was 5.5 ± 0.3 servings (3.5 cups) per day but only 3.9 ± 1.5 (2 cups) when fruit juice and French fried potatoes were excluded. Total FV intake (excluding French fries and fruit juice) was inversely related to several markers, ($P \leq 0.05$ except where indicated), CRP ($P \leq 0.01$), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) in plasma, and F2-isoprostanes, but not PGF2α, in urine. Fruit intake alone was correlated with reduced CRP and interleukin-6 (IL-6) in plasma, and vegetable intake alone with reduced TNF-α, and vegetables and legumes with lower IL-6. French fries and fruit juice were not correlated with any biomarker.

The available data related to inflammatory markers is promising and suggests a mechanism by which FV intake may be protective in a variety of conditions in humans.

**Oxidation**

Oxidative stress is associated with chronic disease and has been widely studied. Recent data have shown that oxidative processes are dependent on the physiologic milieu, including the presence or absence of underlying disease. A study in Sweden demonstrated that women with diabetes were less likely to be protected by FV intake than healthy controls. A group of 95 healthy women and 62 women with diagnosed CVD (71% of sample), diabetes, and/or cancer were randomly selected from the Swedish Mammography Cohort.[47] Quartiles of FV intake were determined on the basis of a 96-item FFQ. Healthy women in the highest vs. lowest quartile of FV intake (650 vs. 520 g/day) had significantly lower levels of plasma extracellular superoxide dismutase (EC-SOD, $P<0.001$). EC-SOD is an endogenous antioxidant that scavenges superoxide radicals and is thus indicative of oxidant stress. In the unhealthy women there was no association between FV intake and EC-SOD across quartiles of FV intake, suggesting that the presence of disease modulates the interaction between antioxidants and FV.

## COGNITION

Advancing age is accompanied by declining cognitive ability and increased incidence of neurodegenerative diseases such as Alzheimer's. Behavioral and neuronal decline is thought to be partly due to increased vulnerability to oxidative and inflammatory stressors associated with aging.[48] Lifestyle interventions that mitigate these processes have been associated with improved performance and may even slow down cognitive decline. Detailed reviews of the potential mechanisms associated with age-related decline and neurodegeneration and the potential benefits of dietary factors, including FV (primarily in animal models), have been published.[48, 49] A number of recent observational studies in humans have added to the data suggesting that FV intake is associated with improved cognitive function.

The Whitehall II study assessed more than 5000 office workers in London, England, for several health-related behaviors, including frequency of fresh FV consumption, and tested for an association between these and cognitive function over a period of 17 years.[50] Tests of executive cognitive function (reasoning, verbal fluency) and a memory assessment were conducted in year 17. Subjects who reported consuming fewer than 2 FV/day compared to more than 2/day were at a higher risk for poor cognitive function at three follow-up time points in the study, including study baseline (mean age of cohort 44 years, 32% higher odds); 11-year follow-up (mean age 56, 60% higher odds); and 17-year follow-up (mean age 61, 85% higher odds). Reduced FV intake tended to be associated with poor function on memory tests. Health behaviors and FV intake were assessed every two years during the study, and there was a cumulative risk associated with low FV intake over time. Subjects who reported <2 servings FV/day more often over the 17-year follow-up had nearly 2.5 times the risk of poor cognitive function (P<0.0001) and 38% higher risk for poor memory (P<0.02). When low FV intake was combined with other unhealthy behaviors, including smoking, low physical activity, and alcohol abstinence, the odds increased by 3- and 2-fold for poor cognition and memory, respectively. An important limitation to this study is the use of a single question to determine fresh FV intake. However, the findings are consistent with other data showing that diet and health-related behaviors in early life influence subsequent cognitive function. The extended follow-up period and individual functional assessments are strengths of the study.

**A number of recent observational studies in humans have added to the data suggesting that fruit and vegetable intake is associated with improved cognitive function.**

Another prospective study of shorter duration reported similar findings.[51] A group of 1433 subjects, baseline mean age of 72.5 (±5.1), were followed for 2.7 to 7.6 years (median 7.3). Self-reported intake of fewer than 2 servings of FV/day was associated with a 26% higher risk of incident mild cognitive impairment or dementia (P=0.04). These data were based on a cohort of randomly drawn subjects living in Montpelier, France, who were examined by a neurologist every two years as part of an ongoing neuropsychiatric cohort study. The goal of the study was to determine a predictive model for dementia. Based on the findings, FV consumption was an important predictor, along with diabetes, depression, apolipoprotein ε4 allele, and crystallized intelligence (based on reading test scores).

A cross-sectional study conducted in Norway demonstrated a dose-response relationship between FV intake and cognitive performance in an elderly subset of subjects (n=2031, 70-74 years, 55% women) enrolled in the ongoing Hordaland Health Study.[52] A validated, 169-item FFQ was self-completed to assess dietary intake in the year prior to completing 6 tests of cognitive performance. Average intake of total FV was 400 g/day, including 240 g of fruit. Performance on all cognitive tests was significantly higher in subjects who consumed >144 g/day than those who reported less or no intake of FV (P<0.01 on 5 tests; P=0.06 on visual-spatial skills), after adjustment for multiple confounders. In fully adjusted models, there was a dose-dependent significant improvement on 4 of 6 tests, with increasing intake of total FV up to 500 g/day and for vegetables up to 150-200 g/day. An extensive analysis of the correlation suggested that several individual FV were positively associated with individual test scores.

Another cross-sectional study in healthy adults aged 45-102 years showed a positive correlation between high FV intake and performance on several tests of cognition and memory.[53] Healthy German adults (n=193) completed neurologic and physical tests, e.g., an ECG, blood pressure, blood levels of routine parameters, lipophilic antioxidant vitamins, and markers of anti-oxidant stress, as well as 3 neuropsychological tests which included screening for dementia. Dietary assessment was based on a modified qualitative FFQ to determine FV intake in the 2 weeks prior to testing. Subjects in the high FV group (n=94) consumed >350 g FV/day compared to those with low intake of 0-100 g/day but were comparable in age, body weight, and lifestyle habits. The analysis showed favorable performance on all neuropsychological tests in the high versus low FV group (P=0.002 to 0.0001 across tests). There were also significantly higher plasma levels of the oxidative stress marker 8,12-isoprostane F2α-VI as well as elevated β-carotene, α and γ-tocopherol, and several carotenoids, except for lutein and retinol, in the high versus low FV group. The beneficial effects on cognitive performance were independent of age, such that older subjects (>90 years) in the high FV group had better scores than younger subjects in the low FV intake group.

There are two recent reports of the association between FV and neurodegenerative disease/decline in aging adults. An analysis of data from registered sets of twins in Sweden was completed to determine if FV intake, based on a questionnaire completed 30 years prior, might be associated with later risk of dementia.[54] It was found that subjects who reported at baseline that FV were a "medium or great part" of their diet had reduced odds of developing dementia (27% lower, P=0.05) and Alzheimer's disease (40%, P<0.01) 30 years later, compared to those who reported that FV had a "small or no part" of their diet. In a stratified analysis the reported inverse association between FV and these conditions was significant only for women and for subjects who reported angina. The authors speculated that longevity in women or increased susceptibility to oxidative stress might account for the influence of gender, whereas angina was likely associated with higher risk due to vascular factors. The authors acknowledged the limitation of using a single baseline assessment completed 3 decades before the analysis and the potential that dietary intake of FV and other risk factors might be modified in the interim. However, the rare opportunity for a 3 decade follow-up period and consistency with the results of other studies related to FV and cognitive function is encouraging.

A prospective study published in 2006 suggested the fruit juice was protective against the development of Alzheimer's disease over 6.3 years of follow-up.[55] A cohort of 1589 Japanese Americans was interviewed and completed a semi-quantitative FFQ with images to assess dietary intake. A strong inverse association between fruit juice consumption and Alzheimer's disease was found, accounting for a 76% reduced risk after adjustment for other variables in subjects who drank juice 3 times/week compared to less often than once a week. The protective effect appeared in all strata of education, smoking status, apo E phenotype, and total fat intake. While this study is intriguing, much work remains to confirm the findings.

The potential of FV intake to impact cognitive performance in humans is an exciting new area that has extended beyond mechanistic and animal studies. The study of cognition in healthy subjects and cognitive and neurodegenerative decline with aging is complex and will require sophisticated, long-term studies to determine if FV are consistently protective.

## DIABETES

Type 2 diabetes has been on the rise over the past two decades with an estimated global prevalence of 6.4%. Observational studies suggest that FV consumption may be inversely associated with risk of diabetes.

A meta-analysis published in 2007 included 5 prospective cohort epidemiologic reports published prior to 2005 and found no significant effect between FV consumption in association with type 2 diabetes.[56] However, recent trials have provided more promising data, suggesting a possible link.

A recent analysis of the EPIC-Norfolk cohort examined the association between FV intake and incident diabetes over a period of 12 years of follow-up.[57] A total of 21,831 middle-aged, moderately overweight (average BMI = 26) participants in Norfolk, England, were assessed for dietary intake in the prior year using a 130-item, semi-quantitative FFQ adapted from the U.S. Nurses' Health Study. During follow-up, 735 cases of diabetes were identified (423 men; 312 women). Multiple, logistic regression models demonstrated an average 22% reduction in diabetes risk after adjustment for confounders. The reduction was more pronounced for fruit alone, 30% vs. 20% for vegetables. The odds ratio (OR) was 0.70 for fruit and 0.80 for vegetables for the highest versus lowest quintile of intake (95% confidence intervals, 0.54-0.90 and 0.62-1.03, respectively).

**Trend data from a recent 2010 meta-analysis suggested a beneficial effect of fruit and vegetable intake related to incidence of diabetes. Consuming 1.35 servings of green leafy vegetables compared to 0.2 servings per day was associated with diabetes risk reduction of 14%.**

One potential mechanism by which fruits and vegetables might be protective is through antioxidant activity associated with vitamin C or phytochemicals. In this study, there was a strong negative association between plasma levels of vitamin C and risk of developing diabetes (62% reduction), but plasma vitamin C was only modestly correlated with FV intake. The authors noted that some FFQs may overestimate FV and therefore attenuate a potential association between their intake, vitamin levels, and disease risk.

Another prospective study of the large, longitudinal prospective Nurses' Health cohort in the United States suggested an association between intake of fruit and green leafy vegetables and lower risk of developing diabetes.[58] Long-term usual diet intake was determined by calculating the cumulative average of FV intake reported on validated FFQs every 2 years over a follow-up period of 18 years. During this time, 4529 cases of diabetes were self-reported. Although total FV consumption was not associated with diabetes, intake of total fruit and green leafy vegetables was inversely related to the development of the disease after adjustment for several independent risk factors. There was a significant risk reduction of 18-26% associated with a 3-serving-per-day increase of fruit ($P<0.001$) and a 9-12% reduction with a 1-serving-per-day increase in green leafy vegetables (spinach, kale, and lettuces). Fruit juice was analyzed separately in this study and was positively correlated to the development of diabetes, with an 18% higher risk associated with a 1-serving-per-day increase in intake ($P<0.001$). The authors acknowledged the potential for misreporting fruit punches as fruit juices, an important limitation in the study which is based on self-reported dietary data.

A more recent meta-analysis examined 6 studies published between 2000 and 2008, including the studies described in this overview.[59] The composite analysis of these prospective cohort studies included assessment of FV intake related to incidence of diabetes. The combined estimates of hazard ratios or relative risk were pooled and did not show any significant reduction in risk for FV, combined or separately. However, trend data suggested a beneficial effect of FV intake, and consuming 1.35 servings of green leafy vegetables compared to 0.2 servings per day was associated with risk reduction of 14% ($P=0.01$). The meta-analysis included fewer men (only two studies), and the significant heterogeneity between studies prompted the authors to standardize portion sizes, recalculate quantities for quintiles, and incorporate other assumptions that suggest these data be viewed with caution. However, their analysis and others add to the evidence that dietary factors are linked to diabetes and that intake of green leafy vegetables, in particular, might be protective.

## EYE HEALTH

Glaucoma involves progressive deterioration of the optic nerve and is the second leading cause of blindness after cataracts.[60] A combined analysis of prospective data from the Nurses' Health Study and the Health Professionals Follow-up Study published in 2003 did not demonstrate an association between FV intake and risk of glaucoma, but a recent cross-sectional study suggested that consumption of some FV might be protective.[60] A sample of women (n=5482) included subjects participating in the Study of Osteoporotic Fractures from several centers across the U.S. who completed an eye examination after 10 years in the study. Of these, a random sample of women was selected for more extensive assessment including glaucoma photography grading (n=1155, 87.5% White; 12.5% Black; 6.2% with diabetes). The prevalence of glaucoma in the total group was 8.2% and 16.7% in subjects who were 85 years or older (n=150). Dietary data were collected using a commercially available block FFQ. The odds of having glaucoma were decreased by consuming several vegetables, including carrots 2 servings/week (64% reduction, P=0.009), compared to fewer than 1/week. Peaches (canned or dried) and greens (collards or kale) consumed >1/month versus < 1/month also significantly reduced risk by 47% (P=0.04) and 69% (P=0.034), respectively.

**The odds of having glaucoma were decreased by consuming several vegetables, including carrots 2 servings/week (64% reduction), compared to fewer than 1/week. Peaches (canned or dried) and greens (collards or kale) consumed >1/month versus <1 month also significantly reduced risk by 47% and 69%, respectively.**

A case-control study in India investigated the food and nutrient intakes of 140 patients with cataracts compared to 100 controls from low- and high-income groups.[61] A structured FFQ and interview was used to assess dietary intake of nutrients and 94 foods. The analysis indicated that fruit intake was inversely associated with oxidant stress in the blood, as well as markers of opacity and oxidation in the lens. Patients had significantly lower intakes of fruit, green leafy vegetables, and several other nutrients that might be protective against cataracts, leading the authors to suggest that FV and antioxidant nutrients are influential factors and that additional data are needed to establish a conclusion.

Oxidative processes are associated with eye health and likely to be influenced by FV intake. With an increasingly aging population in the U.S., it is important to determine preventive dietary approaches to reduce risk of cataracts and glaucoma.

## PULMONARY HEALTH

Lung tissues are vulnerable to oxidative damage due to high and continual exposure to oxygen. The potential of FV to benefit respiratory health is of particular interest due to their high antioxidant content. Recent investigations have focused on pulmonary function, asthma, and allergy in youth.

A cross-sectional study was conducted on 2112 adolescents living in 13 communities in the U.S. and Canada. Mean age of the subjects was 17.6 (± 0.6) years; 45% were males and 89% were White. Assessment of FV intake was done via a semi-quantitative FFQ.[62] It was found that low fruit intake (<0.25 servings/day) was associated with lower forced expiratory volume in 1 second (FEV1, a common measure of ventilatory function), and there was a 36% increase in self-reported, chronic bronchitic symptoms (P<0.05) compared to teens consuming more than 0.25 servings. Other markers of lung function including forced vital capacity (FVC) were not affected. It is noteworthy that even teens who were at the highest percentiles of consumption were well below the recommended number of servings of FV (1.3 and 3.4 servings for FV, respectively, at the 80th percentile).

A study in Mexico demonstrated that FV intake may reduce adverse effects of air pollutants on pulmonary function in children.[63] Young children with asthma (n=158, median age 9.6 years) and controls (median age 9.3 years, n=50) completed spirometric tests of lung function and were assessed for inflammatory markers and exposure to pollutants. Mothers completed a 108-item FFQ and a FV index score was assigned based on intake and the use of vitamin supplements. Higher intake of FV was associated with a significant 8% reduction in the inflammatory cytokine and interleukin-8 (IL-8) in asthmatic children with the lowest intake of FV, but there was no effect in healthy control children. There was a positive interaction between ozone exposure and FV; the protective effect of FV was significant when ozone exposure was highest, reduced IL-8, (P =0.009) and more likely to improve FVC (P=0.037). Although the dietary analysis in this study is confounded by supplement use (comparable intake between high and low FV consumers), it is plausible that components in FV could attenuate the pro-oxidant and inflammatory effects of environmental pollutants in children with asthma.

A study of children in four rural areas of Crete demonstrated that some FV were associated with reduced asthma and allergic symptoms.[64] Parents of 690 children (aged 7-18 years) completed questionnaires regarding their children's respiratory health and allergic symptoms. Skin prick tests for common aeroallergens were also conducted. Diet was assessed using a 58-item questionnaire, although the analysis for FV included only 8 of the commonly consumed FV with high antioxidant content. It was found that average consumption of >1/day compared to <1/day for several FV reduced the odds of having ever experienced wheezing, ranging from an 86% reduction associated with apple intake ($P<0.01$) to 70% for oranges ($P<0.05$). Fresh tomatoes were the only vegetable associated with reduced risk (68%, $P<0.01$). Grapes were the most consistently protective fruit; average intake of 1 serving/day was inversely associated with ever and current wheeze and current, allergic, and seasonal rhinitis. Allergic rhinitis was also inversely associated with daily intake of orange and kiwi. There was no association between FV intake and atopic allergies. A limitation of this study is the restrictive analysis including limited FV; in addition, only two categories of intake were analyzed for each.

A number of studies searched for a potential association between maternal consumption of FV during pregnancy and pulmonary outcomes in offspring, although the results have been mostly null. A recent study in the UK found no association between self-reported maternal intake of total FV at 32 weeks of gestation, although apple intake was beneficially associated with reduced wheeze and asthma in their offspring at 5 years of age (n=1209).[65]

A study of 763 maternal-child pairs in Japan assessed maternal intake using a semi-quantitative diet history questionnaire. There was no link between maternal consumption of total FV intake and wheeze in infants at ages 16-24 months, and no protective effect of apple intake.[66] Similarly, a longer-term study in the Netherlands, including over 4,000 pregnant women, did not find a link between FV intake during pregnancy and childhood asthma outcomes in children during 8 years of follow-up.[67]

In conclusion, recent data continue to suggest that FV intake might be associated with lung function in a beneficial manner, although there is clearly a need for well-controlled clinical trials to further define the link.

### *Chronic Obstructive Pulmonary Disease (COPD)*

COPD is characterized by airway inflammation as well as oxidative processes that play a role in the course of the airflow obstruction associated with the disease. It is proposed that the bioactive components with anti-inflammatory and anti-oxidant activity in FV might be effective in improving the course and risk of COPD. This theory has been tested in 3 recent studies.

A 3-year-study was conducted on clinic patients diagnosed with COPD to determine if fresh FV consumption might be associated with improved pulmonary function, assessed by post bronchodilation (FEV1).[68] Patients living in Greece (88% males) were randomized to either a FV group (n=60) in which they were instructed to increase daily consumption by ≥1 serving FV per day or maintain a standard diet (n=60). The FV group reported a significant increase in FV intake ($P<0.001$) based on completion of a dietary questionnaire during clinic visits every 6 months for 3 years. The FV group also experienced a significant annual improvement in FEV1 ($P<0.01$)

and improved spirometric values by the end of the study, including FEV1, FVC, and FEV1/FVC, compared to patients in the control group who experienced a decline in lung function. The authors postulated that the antioxidants in FV might have accounted for the effect, although it is noteworthy that the meat consumption score also declined in the FV group, and compliance measures in this study were limited to self-reported data.


A case-control study in Turkey included 40 male patients diagnosed with COPD compared to 36 matched healthy smokers. Data were obtained from a face-to-face interview and an adapted FFQ with photographs to assess food intake in the preceding 12 months.[69] Estimated food intake scores based on portion size and frequency were assigned for several food categories, including FV which was significantly higher in healthy controls versus cases (P<0.001). FV intake was also among the few independent dietary predictors of COPD (0.039) in this study.

A more recent case-control study in Japan also found that mean intake of FV differed between cases and controls. Patients with COPD diagnosed within 4 years of the study (n=278, 88% males) consumed significantly fewer fruits (P<0.001) and vegetables (P=0.002) than controls (n=400, 83% males).[70] Dietary intake was based on an interview and a 138-item FFQ to determine consumption patterns 5 years prior to the study and onset of COPD. Logistic regression analysis showed a 38% lower risk of COPD between subjects in the highest quartile of vegetable intake (>252 g/day) compared to the lowest quartile of intake, <111 g/day (P for trend = 0.037). There was also a 51% reduction in reported breathlessness (P=0.02). An inverse association with fruit intake was present but was not statistically significant.

Case-control studies are subject to recall bias in subjects reporting dietary intake after a clinical diagnosis. However, there is enough consistency in this work to suggest that further work is warranted to explore the potential benefit of FV on the course and risk of COPD.

## SKIN HEALTH

A study of 763 maternal-child pairs in Japan assessed maternal diet using a semi-quantitative diet history questionnaire to determine if prenatal intake affected pulmonary and skin health in offspring. There was no link between maternal consumption of total FV intake and tested outcomes in infants at ages 16-24 months, although citrus fruit intake was inversely linked to eczema by 47% (P=0.03 for trend), and this finding remained significant after adjustment for plasma levels of antioxidants.[66] Fruits and vegetables have been implicated indirectly with tissue health through anti-oxidant processes, although this preliminary finding remains to be confirmed.

## WEIGHT AND OBESITY

Given the increasing rate of obesity, there is a great deal of interest in establishing the dietary variables associated with weight maintenance or weight loss. Fruits and vegetables as a group are low in energy density, high in water content, and a significant source of dietary fiber. Several studies have tested the hypothesis that increased FV promote weight loss or improve body weight regulation.

One large-scale prospective study included 89,432 subjects (58% women) from 6 cohorts (5 countries) in the EPIC from sites in Denmark, Germany, Italy, the Netherlands, and the UK.[71] The median intake of FV, as determined by FFQs and 24-hour recalls, was 324 g/day in women and 377 g/day in men, although there was a wide range between countries. During the follow-up period of 6.5 years, there was a small average weight gain of 330 g/year. The analysis showed that FV intake was modestly related to smaller weight gain; for each 100 g of FV consumed there was a 14 gram lowering of predicted weight gain per year. In a separate analysis of subjects who quit smoking and were more prone to weight gain, each 100 g increase in FV reduced predicted weight gain by 37 g/year. Although this was a larger trial, the use of a single baseline assessment of dietary intake assumes that there was no change in FV over the course of the study which is a significant limitation.

A randomized controlled trial in Brazil also sought to quantify the effects of FV on body weight regulation in 80 men and women (46.5 ± 9.5 years, 76.3% women, 82.5% White) who participated in a 6-month intervention, including individualized dietary counseling and intensive lifestyle modification.[72] The consumption of several food groups including FV was assessed using a 75-item FFQ and an interview with a nutritionist at baseline and after 6 months. There was a significant reduction in BMI and an increase in FV intake (P<0.05) after 6 months and a significantly negative correlation between BMI and FV intake and total vegetables (P=0.002), fruits (P=0.011), and dark or yellow fruit/vegetables (P=0.035). Several other nutrients were associated with FV intake including β-carotene, folate, and fiber. For each 100-gram increase in total fruit or total vegetables, there was a reduction of 500 g and 300 g of body weight after 6 months. Multiple linear regression models showed that fruits, vegetables, and fiber from total FV accounted for 13%, 14%, and 19% of the observed weight loss, respectively. However, the authors reported a 20% drop-out rate after 6 months, a common problem in weight-loss intervention trials.

One group of investigators completed a cross-sectional study of men (n=5094) and women (n=6613) in Spain to determine the association between FV intake and age-associated weight gain over 5 years.[73] Self-reported dietary FV intake was based on a validated, 136-item semi-quantitative FFQ (13 separate items for fruit and 11 for vegetables). The median age of the subjects across quintiles of dietary intake ranged from 35 to 51 years. Self-reported weight gain in the 5-year-period prior to the assessment was at least 3 kg in 38% of men and 29% of women. BMI was inversely associated with the highest quintile of FV intake for men in all models (P<0.001), but the effect was attenuated after adjustment for fiber (P=0.199). There was a significant inverse association between weight gain and the combined intake of total FV and fiber (odds ratio 0.69; 95% confidence interval of 0.59-0.75, P<0.001). This study was based on self-reported and cross-sectional data but is consistent with other studies that have suggested an inverse association between FV, fiber, and weight gain in adults.

**Although it remains a challenge to determine the independent effect of fruits and vegetables on body weight regulation, the majority of the available data support the recommendation to increase both fruits and vegetables to promote weight loss and help maintain a healthy body weight in adults.**

Another study with a similar goal but a prospective approach sought to determine the association between FV intake and weight gain over a 10-year period using baseline and follow-up data.[74] A cohort of 206 healthy subjects in Valencia, Spain, ranged in age from 15-80 at baseline and gained an average of 3.41 kg over the study period (±.6.9 kg). The risk of weight gain decreased across quartiles of FV intake with a 78% lower risk in the fourth quartile (>.698 g/day) compared to the lowest (<.362 g/day) (P=.0.022 for trend). Significant reductions in risk of weight gain were also observed when fruits or vegetables were analyzed separately. This study and the ones previously cited suggest that higher FV intake is associated with reduced risk of long-term weight gain.

Some trials have focused on fruit consumption only and have found a significant inverse association between BMI and fruit intake in cross-sectional or longitudinal analyses.[75] A review of 16 studies published between 1996 and 2008 included 3 intervention studies, 8 prospective studies, and 5 cross-sectional studies that examined the role of fruit in weight loss or body weight regulation.[76] The bulk of the data showed that fruit intake is inversely associated with body weight, although the authors indicated that there are few studies that solely examined the independent effect of fruit itself.

Although it remains a challenge to determine the independent effect of FV on body-weight regulation, the majority of the available data support the recommendation to increase both fruits and vegetables to promote weight loss and help maintain a healthy body weight in adults.

## SUMMARY AND COMMENTS

Research related to FV over the past 5 years has expanded and added to earlier evidence supporting a positive association between FV intake and human health. Several encouraging trends are noteworthy, including a greater number of investigations being conducted in countries beyond America and Europe to include non-Western groups, demonstrating potential benefits of FV intake across populations. There is also an important shift toward recognizing the value of the composite of nutrients and components in FV, rather than attributing observed outcomes to isolated or single compounds.[77] Several investigators acknowledged that mixtures and interactions in foods are difficult to mimic with isolated compounds, and that native FV are often more effective.

A few new studies have suggested that the variety of FV consumed might be as important as the quantity. Although national guidelines, health professionals, and organizations advocate variety based on nutrient composition, the emphasis in most scientific studies has historically been placed upon quantity.

**Research related to fruits and vegetables over the past 5 years has expanded and added to earlier evidence supporting a positive association between fruit and vegetable intake and human health.**

Provocative new work in humans has built upon animal data suggesting that consuming FV may improve cognitive performance in both healthy individuals and those with neurodegenerative conditions. Promising studies of the effect of FV on disease-related processes, including inflammation and oxidation, advance our understanding of these conditions and others. There is also support for a positive effect of FV on pulmonary function, particularly in COPD, and the potential of FV to attenuate the adverse effects of environmental pollutants on lung health.

A number of new studies have demonstrated that body-weight regulation and related conditions, including diabetes and hypertension, might be positively impacted by FV consumption, an important and timely focus given the need for more effective strategies to promote weight loss. There are promising but inconsistent data related to the effects of FV on bone mass in humans. However, there is still much work to be done to determine the independent effect of FV on health and to expand upon preliminary findings that hint at positive effects of FV intake on arthritis and eye health.

In spite of these findings, a number of large prospective trials have been published and the results have not consistently supported the outcomes of earlier observational and case-control studies, particularly related to cancer, and to a lesser extent, cardiovascular disease. While the impressive numbers of subjects in these studies are important, methodological limitations are still present. Many large trials rely upon self-administered FFQs to determine FV intake at periodic intervals, often 2 or more years apart. Although most assessment tools have been validated, it is possible that there is systematic under- or over-reporting of food groups. Infrequent assessment also increases the chance of missing dietary shifts between measurement periods.

Inconsistent findings related to FV and cancer, cardiovascular disease, and bone health have also been reported in meta-analyses and recent pooled reports. However, as acknowledged by the authors of many of these, there is a significant degree of heterogeneity between studies examining FV intake and human health. A number of assumptions are made when data are pooled to reconcile the great variety of approaches to dietary assessment, inconsistent stratification and classification of FV intake and quantities, diverse outcome measures, highly variable duration periods, and different exposures to FV.

It is important to be cautious in interpreting the outcomes of recent reports and to recognize the need for further work using well designed, tightly controlled and standardized approaches across multiple conditions and populations. There are numerous plausible mechanisms by which FV might be protective and many unanswered questions regarding the potential importance of variety, quantity, duration, and nature of FV effects on disease-related processes. Thus, the study of FV must remain an active area of research to confirm the true effect of FV intake on human health and build upon the promising data currently available.

# Appendix

The different types of studies mentioned in this scientific overview are outlined below. **They are listed in order from strongest to weakest in inherent design.**[78] Also included in this Appendix are definitions of key terms found in this overview and a summary of the limitations of systematic reviews and meta-analysis.

## CLINICAL STUDY TYPES:

### *Experimental Studies*

The allocation or assignment of individuals is under the control of the investigator and thus can be randomized. The key is that the investigator controls the assignment of the exposure or the treatment, but, otherwise, symmetry of potential unknown confounders is maintained through randomization. The randomization also provides a better foundation for statistical procedures than do observational studies.

**Randomized Controlled Clinical Trial (RCT):** A prospective, analytical, experimental study using primary data generated in the clinical environment. Individuals similar at the beginning are randomly allocated to two or more treatment groups, and the outcomes from the groups are compared after sufficient follow-up time. Properly executed, the RCT is the strongest evidence of the clinical efficacy of preventive and therapeutic procedures in the clinical setting.

**Randomized Cross-Over Clinical Trial:** A prospective, analytical, experimental study using primary data generated in the clinical environment. Individuals with a chronic condition are randomly allocated to one of two treatment groups and, after a sufficient treatment period and often a washout period, are switched to the other treatment for the same period. This design is susceptible to bias if carry-over effects from the first treatment occur. An important variant is the "N of One" clinical trial in which alternative treatments for a chronically affected individual are administered in a random sequence, and the individual is observed in a double-blind fashion to determine which treatment is the best.

**Randomized Controlled Laboratory Study:** A prospective, analytical, experimental study using primary data generated in the laboratory environment. Laboratory studies are very powerful tools for doing basic research and exploring mechanisms because all extraneous factors, other than those of interest, can be controlled or accounted for (e.g., age, gender, genetics, nutrition, environment, co-morbidity, strain of infectious agent). The use of animal models also provides these advantages. However, this control of other factors is also the weakness of this type of study. Subjects in the clinical environment have a wide range of all these controlled factors, as well as others that are unknown. If any interactions occur between these controlled and unknown factors and the outcome of interest, which is usually the case, the laboratory results are not directly applicable to the clinical setting unless the impact of these interactions are also investigated.

### *Observational Studies*

The allocation or assignment of factors is not under the control of investigator. In an observational study, the combinations are self-selected or are "experiments of nature." For those questions where it would be unethical to assign factors, investigators are limited to observational studies. Observational studies provide weaker evidence than do experimental studies because of the potential for large confounding biases to be present when there is an unknown association between a factor and an outcome. The symmetry of unknown confounders cannot be maintained. The greatest value of these types of studies (e.g., case series, ecologic, case-control, cohort) is that they provide preliminary evidence that can be used as the basis for hypotheses in stronger experimental studies, such as randomized controlled trials.

**Cohort (Incidence, Longitudinal Study) Study:** A prospective, analytical, observational study, based on data, usually primary, from a follow-up period of a group in which some have had, have, or will have the exposure of interest, to determine the association between that exposure and an outcome. Cohort studies are susceptible to bias by differential loss to follow-up, the lack of control over risk assignment and thus confounder symmetry, and the potential for zero-time bias when the cohort is assembled. Because of their prospective nature, cohort studies are stronger than case-control studies when well executed, but they also are more expensive. Because of their observational nature, cohort studies do not provide evidence that is as strong as that provided by properly executed, randomized controlled clinical trials.

**Case-Control Study:** A retrospective, analytical, observational study often based on secondary data in which the proportion of cases with a potential risk factor are compared to the proportion of controls (individuals without the disease) with the same risk factor. The common association measure for a case-control study is the odds ratio. These studies are commonly used for initial, inexpensive evaluation of risk factors and are particularly useful for rare conditions or for risk factors with long induction periods. Unfortunately, due to the potential for many forms of bias in this study type, case-control studies provide relatively weak evidence even when properly executed.

**Cross-Sectional (Prevalence Study) Study:** A descriptive study of the relationship between diseases and other factors at one point in time (usually) in a defined population. Cross sectional studies lack any information on timing of exposure and outcome relationships and include only prevalent cases.

**Ecologic (Aggregate) Study:** An observational, analytical study based on aggregated secondary data. Aggregate data on risk factors and disease prevalence from different population groups is compared to identify associations. Because all data are aggregate at the group level, relationships at the individual level cannot be empirically determined but are rather inferred from the group level. Thus, because of the likelihood of an ecologic fallacy, this type of study provides weak evidence.

## DEFINITIONS

### *Prospective Study (Data)*

Data collection and the events of interest occur after individuals are enrolled (e.g. clinical trials and cohort studies). This prospective collection enables the use of consistent criteria and avoids the potential biases of retrospective recall. Prospective studies are limited to those conditions that occur relatively frequently and to studies with relatively short follow-up periods so that sufficient numbers of eligible individuals can be enrolled and followed within a reasonable period.

### *Retrospective Study (Data)*

All events of interest have already occurred and data are generated from historical records (secondary data) and from recall (which may result in the presence of significant recall bias). Retrospective data is relatively inexpensive compared to prospective studies because of the use of available information and is typically used in case-control studies. Retrospective studies of rare conditions are much more efficient than prospective studies because individuals experiencing the rare outcome can be found in patient records rather than following a large number of individuals to find a few cases.

### *Systematic Review*

A systematic review aims to provide an exhaustive summary of literature relevant to a research question. The first step of a systematic review is a thorough search of the literature for relevant papers. The methodology section of the review will list the databases and citation indexes searched, such as Web of Science and PubMed, as well as any individual journals. Next, the titles and the abstracts of the identified articles are checked against pre-determined criteria for eligibility and relevance. Each paper may be assigned an objective assessment of methodological quality. Systematic reviews often, but not always, use statistical techniques (meta-analysis-see below) to combine results of the eligible studies or at least use scoring of the levels of evidence, depending on the methodology used. A systematic review aims to use an objective and transparent approach for research synthesis, while minimizing bias. (See limitations below.)

### *Meta-Analysis*

In statistics, a meta-analysis combines the results of several studies that address a set of related research hypotheses. In its simplest form, this is normally by identification of a common measure of effect size, for which a weighted average might be the output of a meta-analyses. Here, the weighting might be related to sample sizes within the individual studies. More generally there are other differences between the studies that need to be allowed for, but the general aim of a meta-analysis is to more powerfully estimate the true "effect size," as opposed to a smaller "effect size" derived in a single study under a given single set of assumptions and conditions.

## LIMITATIONS OF SYSTEMATIC REVIEWS AND META-ANALYSIS

Although well conducted systematic reviews and meta-analyses can be among the highest form of evidence, the importance of including similar, well-conducted, randomized controlled trials in the review is critical and often overlooked in current reports.[79] Another potential limitation is selection bias; studies with dramatic results have a greater likelihood of being identified and included in the analysis compared to studies of smaller effects. Combining studies of diverse design, quality of methods, and differences in the nature of intervention and subjects is controversial and may lead to a false sense of precision and misleading conclusions.[79,80] A disparity between the results of meta-analyses and subsequent large, randomized controlled trials has occurred in approximately one-third of cases.[79]

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