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The effect of green leafy and cruciferous vegetable intake on the incidence of cardiovascular disease: A meta-analysis

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Abstract

Does the consumption of green leafy vegetables including cruciferous vegetables significantly reduce the incidence of cardiovascular disease? This research question was answered via employing the statistical methods of meta-analysis by synthesizing relevant worldwide studies that address the association between the consumption of green leafy vegetables and risk of incidence of said diseases. All meta-analysis calculations included determination of effect sizes of relative risk, and their respective 95% confidence intervals, heterogeneity of the studies, relative weights for each study, and significance (p) for each study. Eight studies met the inclusion criteria, which investigated the relationship between the incidences of total cardiovascular disease with the intake of green leafy vegetables. The overall effect size (random effect model) was: RR = 0.842 (95% CI = 0.753 to 0.941), $p = 0.002$, which indicates a significant 15.8% reduced incidence of cardiovascular disease.

Keywords

Green leafy vegetables, cruciferous vegetables, meta-analysis, effect size, Forest plot, cardiovascular disease

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Introduction

Why choose the topic of green leafy vegetables (GLV) intake may reduce the incidence of cardiovascular disease (CVD)? There is a need to research the worldwide scholarly journals (mostly peer reviewed) to investigate case–control studies, prospective cohort studies, and comparative studies dealing with GLV intake and the incidence of these human diseases. A meta-analysis can be used to investigate the effects of daily GLV intake on incidence of CVD, not just in the United States but worldwide. This study was novel because it was the first meta-analysis focused solely on GLV including cruciferous vegetables (CV) intake and how they influence incidence of CVD. CV are from the family Cruciferae which are widely cultivated, with many genera, species, and cultivars being raised for food production such as cauliflower, cabbage, cress, bok choy, broccoli, kale, collard greens, and similar GLV and their roots. Meta-analysis studies are feasible where just one researcher can search for relevant studies from computer search engines, reference lists from studies, emails to journals and researchers in this field of study, and physical visits to local college libraries for review and copying of full-text studies. However, meta-analysis research demands a significant amount of time by the meta-analysts to collect and manage effect sizes throughout an investigation, and be efficient in that only relevant studies be used in meta-analysis.

After reading the ''Results'' section of many previously published articles on this topic, there are apparent contradictions in research findings on whether GLV intake does significantly lower incidence of CVD. This meta-analysis research approach attempted to fill this knowledge gap by combining risk ratios (RR) from multiple relevant studies to a common effect size of RR and statistically examine relations between study characteristics and findings. Risk ratios and relative risk have the same meaning and many researchers use these two effect sizes interchangeably. Findings between these different studies were compared by transforming the results into a single common effect size of RR to better understand

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these apparent contradictions in prior research findings. Also, this meta-analysis has wider implications in scientific research by assembling the latest information from several epidemiological and experimental studies on the agents found in GLV that theoretically lower incidence CVD. Many relevant theories on the mechanisms behind these protective effects will be located in one convenient location for all to study.

Problem statement

The problem is that people worldwide are risking their health by not consuming enough GLV on a daily basis. In several studies, many nutrients in GLV, such as dietary fiber, potassium, and antioxidants, have been associated with reduced risk for CVD.[1] What could happen if we do not solve the problem? The World Health Organization (WHO) write on their website that an estimated 17.3 million people died from CVD in 2008, representing 30% of all global deaths.[2] Case–control studies, prospective cohort studies, and comparative studies results have indicated that consuming enough GLV on a daily basis would decrease these deaths rates.[3] These problems are not totally fixable, but the reductions in mortality could probably be reduced by adequate GLV intake. More studies such as this one could encourage the WHO and the Centers for Disease Control and Prevention to spend extra capital on educating people worldwide on the importance of the daily intake of GLV.

Purpose of the study

The goal of this meta-analysis research study was to investigate the relationship of CVD with the intake of GLV. This goal was answered by researching studies that address the association between the consumption of GLV and risk of incidence of CVD. The effect of GLV intake on risk of incidences of disease can be examined by a common measure of effect size such as a hazard ratio (HR), odds ratio (OR), or relative risk (risk ratio) (RR). A meta-analysis combines the results of several studies that address a set of related measurements of effect sizes.[4] Advantages of meta-analysis are to increase validity of research by applying objective formulas to synthesize data across studies rather than using data from a single study, control for between study variation, and make it possible to show if a publication bias exists.[5]

Main variables under study

Source studies

The dependent variables (DVs) of the source studies are the incidence of diseases, and the independent variables (IVs) are the levels of intake of GLV. The incidence of diseases collected in this meta-analysis study includes the following:

DV—Incidence of CVD. Studies that meet all the inclusion criteria, investigated the relationship of CVD with the intake of GLV, and had a common effect size were combined in a meta-analysis. CVD principally refers to cardiac disease and peripheral arterial disease and usually caused by atherosclerosis (plaque buildup in arteries) and/or high blood pressure. In this meta-analysis study, CVD included myocardial infarction, ischemic stroke, coronary heart disease, and ischemic heart disease. The purpose was to investigate a possible tendency of a significant reduction in risk of developing CVD due to frequent intake of GLV.

IV—Intake levels of GLV. Leaf vegetables, greens, vegetable greens, leafy greens, or salad greens, are plant leaves eaten as a vegetable. They come from a very wide variety of plants all over the world, with nearly 1000 species of plants with edible leaves are known. GLV most often come from short-lived herbaceous plants such as lettuce and spinach. The IV of the source studies was an adequate daily intake of GLV and how this influences the DV which was the incidence of CVD. This study recognized the many theories of how GLV intake may reduce incidence of disease. The calculated effect sizes of GLV intake on disease incidence from each study were combined. Meta-analysis: The output variables from the source studies were their calculated effect sizes (one for each source study), which was risk ratio (RR), and were synthesized via meta-analysis methods, to derive the overall effect size for CVD.

Measured adequacy intake of GLV

Diet in these types of source studies was usually assessed by item semi-quantitative food frequency questionnaire that included fruit and vegetable items. For each food item, a standard serving size was specified. Weight or volume of that item was commonly consumed by the US population at one meal was used. On the dietary questionnaires, participants reported their average intake of the specified portion size (serving) for each food over the past year. For each food item on the questionnaire, several responses were possible, ranging from ''never or less than once per month'' to ''six or more times per day.'' Frequencies and portions for the individual food items were converted to average daily intake of each fruit and vegetable item for each participant. The average daily intakes of individual food items were combined to compute total fruit and vegetable intake and intakes of composite fruit and

vegetable groups in most source studies. The consumption of GLV (grams/day) was calculated from these responses; however, intake was not measured consistently for all source studies. Finally, the association between risk for incidence of disease and the highest versus lowest tertile, quartile, or quintiles intake of fruits and vegetables was estimated.

Theoretical assumptions and conceptual framework

Figure 1 describes the IV as an adequate daily intake of GLV collected from source studies and how this influences the DVs which are the incidence of CVD from these source studies. This study recognized the many theories of how GLV intake reduces incidence of disease. The intervening variable facilitates a better understanding of the relationship between GLV intake and reduction of disease. Some of these hypothesized intervening variables found in GLV are folic acid, the antioxidants beta-carotene and vitamin E, soluble fiber, calcium, and vitamin K. It has been theorized in numerous studies that these essential nutrients and phytochemicals found in GLV, if consumed in adequate amounts, reduces the incidences of some human diseases.[6]

The researchers in these studies theorize on the mechanisms of disease reduction caused by GLV intake. In the 2010 decade, researchers are conducting extensive research studies to discover phytochemicals connections to disease prevention, but so far, solid evidence is mostly lacking.[7]

There are thousands of these phytochemicals in GLV, and researchers are just beginning to understand and theorize how a handful of these phytochemicals work, and what is current in the 2010 decade may change tomorrow.[7] There is a need to research the scholarly journals to investigate case–control studies, prospective cohort studies, and comparative studies dealing with GLV intake and the incidence of CVD. A meta-analysis can be used to investigate the long-term effects of daily GLV intake on incidence of CVD. In each of these studies, the IV is the intake level of GLV; the intervening variable is the levels of the elements; the DV is the incidence of disease; and the statistical output is the RR calculated effect size.

Methodology

Most primary studies that investigate the relationship between GLV intake and incidence of human disease report HR, OR, or risk ratio (relative risk), and their 95% confidence intervals for their effect sizes. Unfortunately, with these common reported results, the three effect sizes cannot be converted into each other, and thus the power of this meta-analysis could be reduced. Searching for numerous relevant studies with these common effect sizes was the goal of this meta-analysis because the outcome is dependent and based on the quality and success of an assiduous search for potential studies.[4] Searching for relevant studies was primarily performed by computer search engines seeking databases which included information about the subject. PubMed Central, Academic Search Complete, Medline, Proquest Central, ScienceDirect, Google, and Yahoo online were the most online periodical databases used. Reference lists from studies, physical visits to libraries for review, and copying of full-text studies were also used to obtain as much relevant data as possible, and the use of interlibrary loan from the local community college was used to collect full-text scholarly journal studies. A conservative

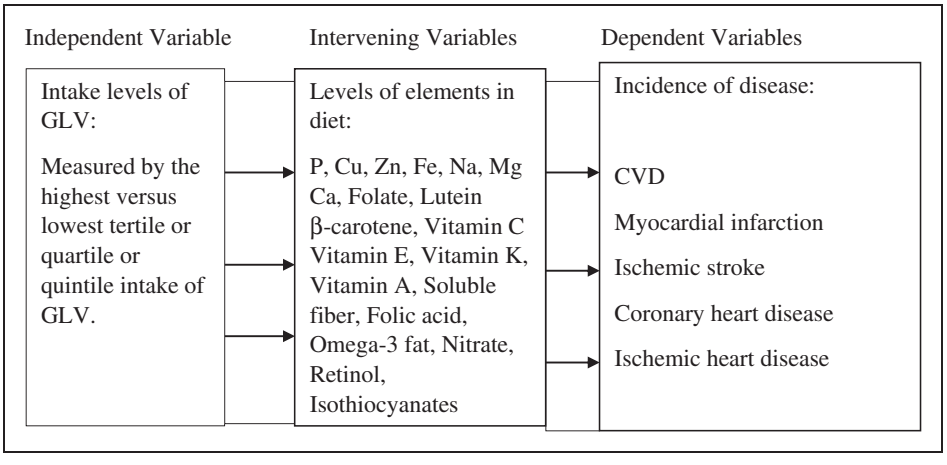

Figure 1. Theoretical assumptions showing relations between intake levels of GLV, intervening variables, and incidence of disease. GLV: green leafy vegetables; CVD: cardiovascular disease.

method for including studies in this meta-analysis was incorporated by reviewing the full-text publications from scholarly peer-reviewed journals to ensure that sound statistical methods were followed, whereas non-scholarly journals were not accepted. Also, source studies were the unit of analysis with only one effect size computed for each source study.[4]

Selection of final set of relevant studies

All studies collected were assigned a unique identification number and compiled into a master database within a Microsoft Office Excel spreadsheet. This allowed for convenient repetitive sorting and extracting of data, and later on for transferring data to supporting statistical compatible software packages.[4] The final set of studies were selected from those studies that met all the inclusion criteria. The main criteria for including an individual study in this meta-analysis were as follows: did the study include the relationship between GLV intake and incidence of CVD and did the researchers from these individual studies include risk ratio and their 95% confidence intervals in their result section.

Inclusion/exclusion of source studies

The criteria for including studies in this meta-analysis included (1) a time period for collecting source studies which was from 1989 until 2014; (2) include only full-text scholarly journal studies; (3) only studies that were published by peer-reviewed and scholarly journals were included; (4) the collection of primary studies had to be a collaborative cohort, case–control, population-based cohort, or a prospective cohort study design; (5) only include relations between similar IVs (GLV intake including CVs) and DVs (incidence of CVD); and (6) studies that reported an effect size of RR and their respective 95% confidence interval data.

Data analysis and statistical testing

All meta-analysis calculations were performed by the software package Comprehensive Meta-Analysis Version 2 by Biostat (CMA v.2). CMA v.2 was developed specifically for use in meta-analysis. These calculations include determining effect sizes (risk ratio (relative risk), and their 95% confidence intervals), heterogeneity of the studies, relative weights for each study, significance (p) for each study, and for determining methods for detecting the presence of publication bias and assessing its impact on the analysis. Risk ratios were calculated in the source studies, whereas the overall effect sizes were calculated by CMA v.2 software. Relative risk (RR) is defined as ratio of the incidence rate among exposed individuals to the incidence rate among unexposed individuals and is used in cohort study design because these designs have a well-defined population. To calculate a crude RR, researchers would use the following formula: $RR = (a/a + b)/(c/c + d)$, where: a = number of individuals with a disease who were exposed, b = number of individuals without a disease who were exposed, c = number of individuals with a disease who were not exposed, and d = number of individuals without a disease who were not exposed.

Borenstein et al.[5] write that the selection of a model must be based soley on the question of which model fits the distribution of effect sizes, and when studies are collected from published literature, the random effects model is a more plausible match for the meta-analysis. These same authors write that changing models if the test for heterogeneity is significant is a mistake, and should be strongly discouraged. Determination on which model to use in this study was decided following analysis of the calculated I^2 and Cochran's Q (Q-value) statistics of hetrogeneity. The Q-value is calculated as the weighted sum of the squared differences between individual source study effects and the pooled effect across studies, with the relative weights being those used in the pooling method.[8] Q-value is distributed as a chi-square statistic with number of studies -1 degrees of freedom. The I^2 statistic describes the percentages of variation across source studies that is due to heterogeneity rather than chance.[8] These same authors write that I^2 is an intuitive and simple calculation of inconsistency of studies' results which does not inherently depend upon the number of source studies in the meta-analysis. In interpreting of the Q-value, heterogeneity of the combined studies exists if its p-value is < 0.05. If the p-value of the Q-value < 0.05, then the random effect model should be used for the meta-analysis.[9] If the p-value of the Q-value > 0.05, then the fixed effect model should be chosen for the meta-analysis. The I^2 can also be used to determine which model to use during a meta-analysis. The following has been proposed for interpreting I^2 and used in meta-analysis; low heterogeneity is $I^2 = 25\%$, moderate heterogeneity is $I^2 = 50\%$, and high heterogeneity is $I^2 = 75\%$.[8]

The relative weights for each study were calculated by CMA v.2 software package. Small studies tend to have wide confidence intervals, and large studies tend to have narrow confidence intervals with larger studies given greater percent relative weights.[10] An effect size of 1.00 represents no treatment effect. Whereas when the effect size falls below 1.00, this indicated participants who consumed GLV in the highest quartile were less likely to develop incidence of disease. If the effect size falls above 1.00, this indicated participants were more likely to develop incidence of disease due to GLV intake in the highest intake quartile. The confidence interval bounding each study reflects the precision of

the estimate, with small studies tending to have wide confidence intervals and large studies tending to have narrow confidence intervals.[10] The use of 95% confidence interval in this study was used, so each meta-analysis performed in this study was statistically significant ($p < 0.05$) if and only if the confidence interval excluded the null value of 1.0 for each effect model synthesized.[10] The conventional value of significance level for this meta-analysis was pre-set to an alpha of 0.05.[11]

Subgroups within studies

CMA v.2 allows the meta-analyst to record data by subgroups within the study. Some studies collected in this study used subgroups, e.g., gender, GLV, CV, premenopausal, postmenopausal, never tobacco, ever tobacco, dark GLV, light GLV, cardia cancer, and non-cardia cancer. In this study, it emerged that the effect sizes were comparable for each subgroup, so it was decided to use the study as the unit of analysis. This required calculating a "combined" effect size (utilizing the CMA v.2 software) for subgroups within each study, and imputes the values for the full group which recorded one treatment effect for each study.

Detecting publication bias

To detect the presence of publication bias, all studies used in this meta-analysis were examined using a funnel plot of the natural logarithm of the effect size versus its precision (1/standard error). The plot by precision is the traditional form.[5] The funnel plot graph, Begg and Mazumdar's test for correlation, Egger's test for regression, Duval and Tweedie's trim and fill, and the classic fail-safe method were calculated by CMA v.2 software for detecting the presence of publication bias and assessing its impact on this meta-analysis study. Borenstein et al.[5] define these multiple methods for detecting the presence of publication bias as follows: (1) Duval and Tweedie's trim and fill builds on the key idea behind the precision funnel plot; that in the absence of publication bias, the plot would be symmetric about the summary effect. If there are more small studies on the right than on the left of the mean effect size, the concern is that studies may be missing from the left. Duval and Tweedie's method imputes these missing studies, adds them to the analysis, and then re-computes the summary effect size; (2) Begg and Mazumdar's rank correlation test reports the rank correlation between the standardized effect size and the variances of the effects. If the rank correlation gave a p-value of >0.05, this would indicate no evidence of publication bias; (3) Egger's linear regression method uses the actual values of the effect sizes and their precision. The standardized effect is regressed on precision and if the regression intercept gave a p-value of >0.05, this would indicate no evidence of publication bias; and (4) Classic fail-safe N test computes the number of possible missing studies with the mean effect of zero that would need to be added to the meta-analysis to yield a statistically non-significant overall effect. If the number of missing studies that would bring the p-value to >0.05 is relatively small, then there is indeed cause for concern regarding the strength of the meta-analysis' overall result. If the number is large, the meta-analyst can be confident that the treatment effect size is not nil.

Data analysis and results

Eight studies met the inclusion criteria, which investigated the relationship between the incidences of total CVD with the intake of GLV. Eight studies shown in Table 1 had a similar common effect size (RR), and a random effect model was used to combine results from the eight studies. Figure 2 shows a Forest plot of the eight studies and the random effect model. Subgroups GLV and CV were combined in five of the studies to calculate one treatment effect for each study as shown in Figure 2. The heterogeneity of the eight studies was $Q\text{-}value = 37.473$, $p = 0.00$, $I^2 = 81.32$. The random effect model indicates an overall RR effect size of the "almost every day" highest versus lowest quantile intake category of GLV on CVD as: RR = 0.842 (95% CI = 0.753 to 0.941), $p = 0.002$.

Figure 3 shows a possible presence of publication bias in the eight CVD studies with the studies distributed to the left about the mean effect size. Duval and Tweedie's method imputes missing studies to the right and adjusts new RR = 0.897, 95% CI = 0.803 to 0.99 from the observed values (0.842, 95% CI = 0.753 to 0.941). Begg and Mazumdar's rank correlation p-value (two-tailed) = 0.137, indicating no evidence of publication bias. Egger's linear regression p-value (two-tailed) = 0.033, indicating possible evidence of publication bias. Classic fail-safe N test imputes there would be 78 missing studies that would bring the p-value to >0.05.

Discussion and summary of the study

A noteworthy finding of this study is the protective effect associated with high consumption of GLV including CVs. These vegetables are a characteristic and traditional dietary habit of worldwide populations. It has been previously postulated that this could explain the very low diabetes, CVD, and cancer incidence rates observed in populations that consume these vegetables. The role of diet in the causation of human disease is

Table 1. GLV on CVD—Qualifying studies.

Study	Effect size (RR)	Sample size	Location of study
Hernandez-Diaz et al. (2002)[12]	0.51 (0.24, 1.06)	342	Spain
Hung et al. (2004)[13]	0.94 (0.89, 0.99)	109,635	North America
Josphipura et al. (1999)[14]	0.76 (0.58, 0.99)	114,279	North America
Josphipura et al. (2001)[1]	0.72 (0.64, 0.83)	126,399	North America
Josphipura et al. (2009)[15]	0.92 (0.72, 1.18)	109,788	North America
Rastogi et al. (2004)[16]	0.33 (0.17, 0.64)	1050	India
Larsson et al. (2013)[17]	0.92 (0.81, 1.04)	74,961	Sweden
Mizrahi et al. (2009)[18]	0.79 (0.63, 0.99)	3932	Finland

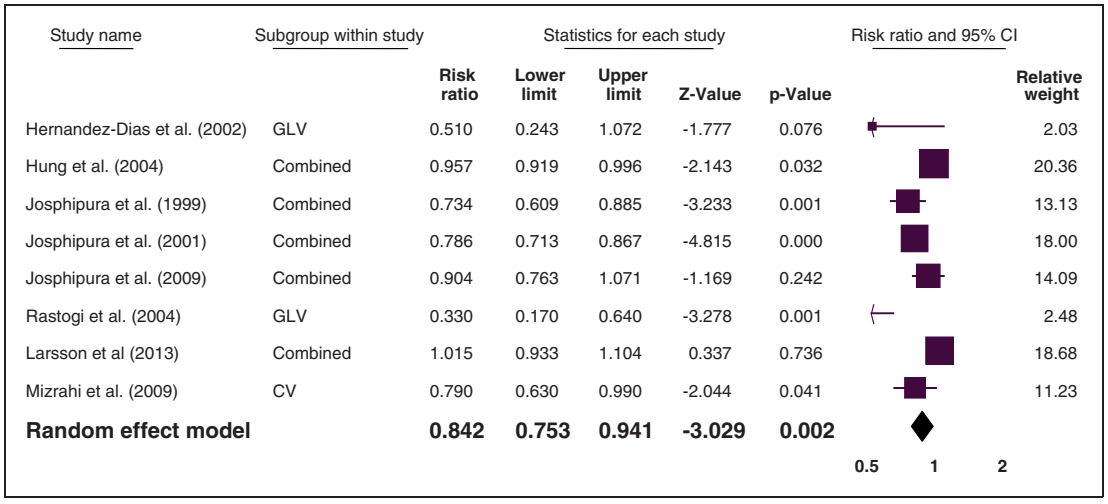

Figure 2. Meta-analysis' Forest plot of GLV intake on CVD incidence.

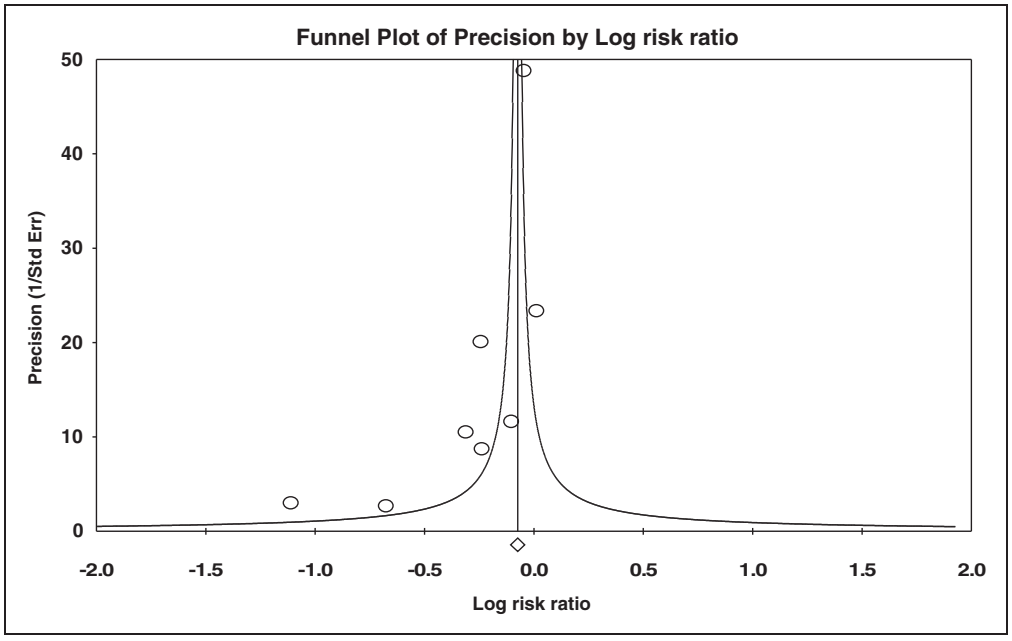

Figure 3. GLV intake on CVD—Publication bias funnel plot.

complex, partly because diet and dietary habits include a wide variety of foods and because the methods by which these habits can be measured are cumbersome as well as difficult to apply to a large number of individuals. This study has been able to provide some clues for further investigation into the role of diet of GLV prevalent in regions where causation of CVD occurs.

In this study, there were only 13 relevant studies found that tested the association between intake of GLV and the incidence of CVD. These included myocardial infarction, ischemic stroke, total stroke, cerebrovascular disease, coronary heart disease, and ischemic heart disease. Eight studies used the effect size RR which included over 540,000 participants including controls in prospective cohort and case–control studies. These eight studies included a total of 26,173 known cases of CVD. The research question of this study was does an increased intake of GLV significantly reduce the incidence of CVD? The random effect model calculated a multivariate RR = 0.842 (95% CI = 0.753 to 0.941), $p = 0.002$ which does indicate an increased intake of GLV significantly reduces the incidence of CVD by 15.8%.

Several agents found in GLV may cause reduction in the incidence of CVD. Bile acid binding capacity has been related to the cholesterol-lowering potential of foods. Lowered recirculation of bile acids results in utilization of cholesterol to synthesize bile acid and reduced fat absorption, thus bile acid binding potential has been related to lowering the risk of heart disease.[19] These same researchers found that steam cooking significantly improved the in vitro bile acid binding of collard greens, kale, mustard greens, broccoli, and cabbage and concluded that GLV, when consumed regularly after steam cooking, would lower the risk of CVD.

Nitric oxide pathway represents a critical advance in understanding CVD, and today a number of human diseases are characterized by nitric oxide insufficiency.[20] The same authors write that regular intake of nitrate-containing food such as GLV may ensure that blood and tissue levels of nitrite and nitric oxide pools are maintained at a level sufficient to compensate for any disturbances in endogenous nitric oxide synthesis. Also, in observational studies, magnesium intake has been inversely associated with hypertension and CVD.[21] The same researchers found the available evidence indicates that dietary magnesium may favorably affect a cluster of metabolic abnormalities including insulin resistance, hypertension, and metabolic syndrome. Metabolic syndrome is prevalent worldwide and is associated with greater risks of major chronic diseases, particularly type 2 diabetes and CVD. As previously stated, GLV are high in magnesium.[22]

Several epidemiological and experimental studies have been published on the cardiovascular benefits of omega-3 fatty acids and that alpha-linolenic acid is an omega-3 fatty acid present in seeds and oils, GLV, and nuts and beans.[23] Antioxidants are important in protection against hypertension, diabetes, CVD, and cancer.[24] Molecular evidence suggests that trace elements and antioxidant molecules found in GLV lower risk of cancer and CVD through mechanisms that modulate free radical attack on nucleic acids, proteins, and polyunsaturated fatty acids.[26,25] Lutein is a member of the carotenoid family, a group of powerful antioxidants, and is a non-provitamin-A carotenoid found in dark GLV. The mechanism by which lutein is involved in the prevention of cardiovascular heart disease may also be related to its role as an antioxidant.[27]

Implications of study

This meta-analysis study found a high daily intake of GLV significantly reduced incidence of several types of CVD. This is the first meta-analysis study that has focused solely on GLV including CV intake and how they influence incidence of these specific human diseases. Relevant studies for the past 25 years were synthesized to demonstrate the importance GLV have in reducing the incidence of these extremely lethal chronic diseases. The study's positive findings should further influence healthcare practitioners, educational instructors of nutrition, and especially government agencies to continuously educate people about the importance of consuming GLV on a daily basis.

Recommendations for further research

Since results from any single epidemiologic meta-analysis study may not provide conclusive evidence; additional studies in other worldwide populations should be done. Further epidemiologic studies will be needed, since large long-term randomized, controlled trials of nutrition would be difficult to pursue to a significant conclusion. Cross and Lim's[28] review of the epidemiologic literature on diet and disease reveals a number of future research directions that should be pursued. These same authors believe overall, more studies are required to resolve inconsistent findings, especially where significant findings are limited to retrospective studies. They write, ''Prospective cohorts would deliver risk estimates less biased by differential recalls by patients or any recent dietary changes due to disease.'' Cross and Lim[28] also state that a thorough ascertainment of data on potential confounders and adjustments are imperative to assess the ''true'' diet-disease associations because this is a common problem of all observational studies. Furthermore, Cross and Lim[28] state that more biomarker studies should be researched

because they may provide more direct evidence for certain nutrients and their interactions with relevant genetic polymorphisms and this may shed light on the potentially complex involvement of nutrition in disease incidence. Thus, this meta-analysis type of study can and should be repeated, once more studies are published.

Further research in the 21st century should be focused on conducting extensive research studies to discover phytochemicals connections to disease prevention because solid evidence is mostly lacking.[7] Researchers are just beginning to understand and theorize how a small percent of the different phytochemicals in GLV work. There are potentially thousands of phytochemical compounds from extracts of plant roots, leaves, and stems that have shown promising potential as anticancer drugs, or for serving as lead compounds in the synthesis of new drugs.[28–30] The potential is here just waiting for new researchers to cure cancer, type 2, and CVD via new phytochemical drug discoveries.

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Ethical approval

Institutional Review Board at the Trident University International.

Guarantor

Dr Richard Pollock.

Contributorship

Theory and editing were improved in this paper by my dissertation committee which included Dr Mickey Shachar, Dr Frank Gomez, and Dr Kyung-Ae Son-Guidry.

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