Review Article

Dietary intakes of green leafy vegetables and incidence of cardiovascular diseases

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Abstract

Aim: Low- and middle-income countries (LMICs) are currently experiencing increasing cardiovascular disease (CVD) rates. Green leafy vegetables (GLV), which are abundant in these countries, are known to be particularly rich in cardioprotective nutrients. This study sought to determine the specific effect of GLV intake on the incidence of CVD.

Methods: Previously published cohort studies on GLV intake and incidence of CVD were retrieved through a systematic search of Google Scholar, EMBASE, MEDLINE, HINARI and Cochrane Library. A methodological evaluation of studies was carried out using the network of Ottawa scale, and a fixed-effect meta-analysis was applied to estimate pooled relative risk (RR) and 95% confidence interval (CI). Heterogeneity was determined using the I^2 statistic. Sensitivity analysis was done using the leave-one-study-out technique. All statistical analysis was carried out at $p < 0.05$ using RevMan 5.4.

Results: The pooled RR (95% CI) of incident CVD events from 17 studies was 0.93 (0.92–0.95). Specifically, GLV intake was inversely related with incident cerebral infarction (RR: 0.92; 95% CI: 0.88–0.96), heart disease (RR: 0.93; 95% CI: 0.87–0.99) and other CVD events (RR: 0.95; 95% CI: 0.93–0.98).

Conclusion: GLV intake was associated with a lower incidence of CVD, and may be a promising primary-prevention strategy against CVD events. The findings are especially important in LMICs where the burden of CVD remains high.

Keywords: green leafy vegetables, cardiovascular diseases, cerebral infarction, coronary heart disease, heart disease, meta-analysis

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Cardiovascular diseases (CVD) account for about 17.9 million deaths annually[1] and a huge burden of health expenditure worldwide.[2,3] Although CVD rates appear to be declining globally,[1,2,4-6] populations in low- and middle-income countries (LMIC)[6,7] continue to experience increasing CVD rates. CVD are preventable and efforts are currently being mobilised to achieve a 25% reduction in mortality rate attributable to CVD by 2025.[8,9]

A promising preventative strategy for CVD is diet.[10-13] However, studies on the potential association of diet and CVD events have

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focused on the effect of red meat,[14,15] salt intake,[16] alcohol,[17] saturated fats/oils and dairy products.[18] Prior reviews and meta-analyses[19-24] investigating the effect of fruit and vegetables on the risk profile for CVD have focused on broad categories of the nutritional modalities. For example, Deng *et al.*[19] and Kwok *et al.*[24] in two reviews of meta-analyses assessed the effect of fruit and vegetable intake, in general, on the burden of diseases and all-cause mortality without providing information on the specific effect(s) of green leafy vegetables (GLV) on the incidence of distinct CVD events.

The information provided by individual studies on the effect of GLV intake remains inconclusive. While some studies reported a reduction in the incidence of CVD events with higher consumption of GLV,[10,25,26] others observed statistically insignificant relationships.[27,28] The pooled effect of GLV intake on incident CVD is currently unknown.

GLV are widely available in LMIC.[29] The vegetables are rich in phytochemicals and micronutrients known to be essential for health.[13,30-32] Also, GLV contain folic acid, vitamins A, C, E and K, as well as high amounts of calcium, iron, potassium, phosphorous and zinc,[33,34] which may be protectively associated with CVD risk.[35] This systematic review and meta-analysis investigated the pooled effect of GLV intake on incident CVD events.

Methods

The systematic review was registered in the international prospective register of systematic reviews and is accessible via https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020181050. Google Scholar, EMBASE, MEDLINE, HINARI and Cochrane Library were searched (in December 2020 using specific search terms independent of language and publication dates) for previously published epidemiological reports on consumption of GLV and CVD. The following search terms were used.

EMBASE, Google Scholar and Cochrane Library search terms: 'vegetables' OR 'chlorophyll-containing vegetables' OR 'green leafy vegetables' OR 'broccoli' OR 'cabbage' OR 'celery' OR 'collard green' OR 'green pea' OR 'lettuce' OR 'spinach' OR 'swiss chard' OR 'turnip green' AND 'cardiovascular disease' OR 'cerebrovascular disease' OR 'cerebral infarction' OR 'cerebral haemorrhage' OR 'coronary heart disease' OR 'heart failure' OR 'subarachnoid haemorrhage'.

MEDLINE and HINARI search terms using PubMed interphases: 'vegetables (Title/Abstract)' OR 'green leaves (Title/Abstract)' OR 'edible green leaves (Title/Abstract)' OR 'green vegetables (Title/Abstract)' OR 'leafy vegetables (Title/Abstract)' OR 'green leafy vegetables (Title/Abstract)' OR 'chlorophyll-containing vegetables (Title/Abstract)' OR 'broccoli (Title/Abstract)' OR 'cabbage (Title/Abstract)' OR 'celery (Title/Abstract)' OR 'collard green (Title/Abstract)' OR 'green pea (Title/Abstract)' OR 'lettuce (Title/Abstract)' OR 'spinach (Title/Abstract)' OR 'swiss chard (Title/Abstract)' OR 'turnip green (Title/Abstract)' AND 'stroke (MesH terms)' OR 'transient ischemic attack (MeSH terms)' OR 'haemorrhagic stroke (MeSH terms)' OR 'ischaemic stroke (MeSH terms)' OR 'cardiovascular disease (MeSH terms)' OR 'cerebrovascular disease (MeSH terms)' OR 'cerebral infarction (MeSH terms)' OR 'cerebral haemorrhage (MeSH terms)' OR 'coronary heart disease (MeSH terms)' OR 'heart failure (MeSH terms)' OR 'subarachnoid haemorrhage (MeSH terms)'. Details of the literature search are in the PRISMA flow chart (Fig. 1).

Study assessment for inclusion and exclusion criteria and data extraction were conducted by two independent assessors (AO and APO) based on the descriptions in the original article. Only studies with usable data and appropriate analytical techniques were included in the meta-analysis. The following information was extracted from each included study: first author name, publication year, sample size, average follow-up time, the incidence of CVD, adjusted relative risk (RR)/hazard ratio and 95% confidence interval (CI), etc.

Studies included in this meta-analysis were prospective cohort reports (where the primary exposure was GLV consumption and outcomes were CVD events) only. Where there are significant levels of data overlap among published studies, the study with complete evidence was included in the quantitative synthesis.

A methodological assessment for risk of bias of included studies was conducted (independently by two members of the review team) using the Newcastle–Ottawa scale for quality assessment of observational reports[36] following the Cochrane Collaboration guidelines.[37]

Statistical analysis

Using the RR and 95% CI for highest quintile/category of GLV consumption compared to the lowest quintile/category of GLV consumption (as reference) for the incidence of CVD events

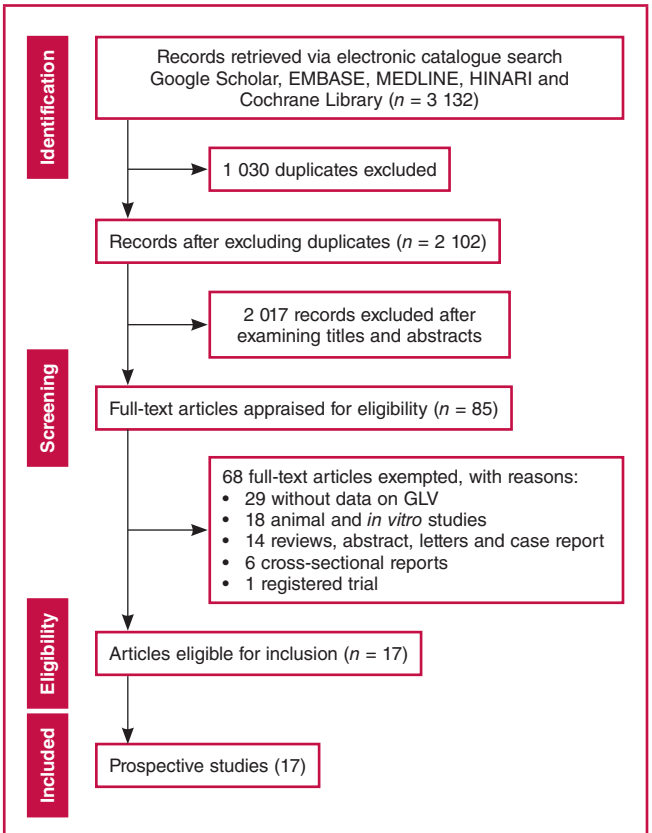

Fig. 1. PRISMA flowchart describing selection of the studies for the meta-analysis

Table 1. Characteristics of prospective reports included in the meta-analysis

	Study characteristics			*Baseline/outcomes evaluation*				
First author	*Year*	*Country*	*GLV intake*	*Incidence*	*Total*	*CVD event(s)*	*Assessment*	*Ascertainment*
Gaziano JM	1995	United States	< 1 s/d* vs ≥ 1 s/d	161	1 299	CVD	Relative-reported deaths[‡†]	Not reported
Joshipura KJ	1999	United States	Increment of 1 s/d[3]	366[W] 204[M]	75 596[W] 38 683[M]	Ischaemic stroke	Self/relative report[‡]	National Stroke Society (NSS) criteria
Joshipura KJ	2001	United States	Increment of 1 s/d[2,3]	1 127[W] 1 063[M]	84 251[W] 42 148[M]	CHD	Self/relative report[‡]	World Health Organisation (WHO) criteria
Johnsen SP	2003	Denmark	1.4 g/d* vs 28.00 g/d	266	54 506	Ischaemic stroke	Self/relative report[‡]	WHO criteria
Sauvaget C	2003	Japan	≤ 1 s/week* vs 1 s/d[2]	1 926	40 349	Stroke	Stroke mortality[‡]	WHO criteria
Hung HC	2004	United States	Increment of 1 s/d[3]	3 864	109 635	CVD	Self/relative report[‡]	NSS criteria
Takachi R	2007	Japan	Not reported	1 386	77 891	CVD	MI or stroke diagnosis using CT scan/MRI[‡]	WHO and NSS criteria
Joshipura KJ	2009	United States	Not reported	1 852[W] 2 040[M]	70 870[W] 38 918[M]	Ischaemic CVD	Self/relative report[‡]	WHO and NSS criteria
Bendinelli B	2010	Italy	≤ 17.60 g/d* vs > 50.80 g/d[1]	144	29 689	CHD	[©]Self/relative report[‡]	Minnesota Code
Oude Griep LM	2011A	Netherlands	34 g/d* vs 105 g/d[2,3]	233	20 069	Stroke	Population and hospital discharge register	Dutch guidelines
Oude Griep LM	2011B	Netherlands	34 g/d* vs 105 g/d[2,3]	245	20 069	CHD	[©]Population and hospital discharge register	WHO criteria
Larsson S	2013	Sweden	< 2.3 s/d* vs > 6.0 s/d[1, 2,3]	4 089	74 961	Stroke	Self report[‡]	Not reported
Bhupathiraju SN	2013	United States	0.22 s/d* vs 1.50 s/day[1, 2]	6 189	71 141	CHD	Self/relative report[‡]	WHO criteria
Rautiainen S	2015	Sweden	< 0.2 s/d* vs > 1 s/d[1,2,3]	3 051	34 319	Heart failure	Heart failure diagnosis and related deaths[‡]	ESC criteria
Wang JB	2016	China	Increment of twice/week	355	2 445	Stroke	Case, pathology, cytology, X-rays, biochemical, ultrasound, endoscopy and surgery reports	Team of reviewers
Buil-Cosiales P	2016	Spain	32·16 g/d* vs 113.00 g/d[1]	342	7 216	CVD	Self/relative report[‡]	Team of reviewers
Blekkenhorst LC	2017	Australia	Intake per 10 g/d	238	1 226	CHD	CHD diagnosis and related death[‡]	Not reported

*Reference group for comparison; [1]energy-adjusted dietary intakes of GLV; [2]additionally adjusted for other intakes, etc; [3]using median values of quintiles; [M]men; [W]women; [©]MI events, coronary revascularisation, or both not preceded by any other CHD event; [‡]authenticated via vital statistics or medical records or designated registry; [†]validated death certificate.
g/d – grams per day; s/d – servings per day; GLV – green leafy vegetables; ESC – European Society of Cardiology; CVD – cardiovascular disease; CHD – coronary heart disease; CT – computed tomography; MI – myocardial infarction; MRI – magnetic resonance imaging.

reported in the included studies, we computed the log of RR and the matching standard error for the overall pooled RR (95% CI) for the incidence of CVD events and by subgroup stratification [cerebral infarction, cerebral haemorrhage, coronary heart disease (CHD), etc.] using an inverse-of-variance method for weighting in all quantitative estimations for dichotomous outcomes.

The degree of heterogeneity was assessed using I^2 statistics assuming a fixed-effect model (where $I^2 < 50\%$) or a random-effect meta-analysis model if $I^2 > 50\%$. The fixed-effect model presupposes the effect size is likely relatively similar across studies in the meta-analysis.[37,38] However, a random-effect model ideates the difference in effect estimates across studies are valid but follows a normal distribution. Publication bias for the likely effect estimate of GLV intake on CVD events was tested using funnel plots.

The constancy of the pooled RR (95% CI) was tested using the leave-one-study-out method (carrying out the meta-analysis several times, excluding a study at a time). All quantitative analyses were conducted at $p < 0.05$ using the RevMan 5.4 software.[39]

Results

Over 3 000 records were retrieved from the literature search in Google Scholar, EMBASE, MEDLINE, HINARI and Cochrane Library but 1 021 duplicates were excluded. Also, 2 011 records were excluded after screening the titles and abstracts (Fig. 1). On full-text assessment, 65 records were excluded and 17 prospective reports (five reports on composite CVD events,[10,25-27,40] five reports on coronary heart disease,[28,41-44] one report on heart failure[45] and six reports on stroke[46-51]) were included in the meta-analysis.

Table 2. Methodological assessment of prospective studies using the Newcastle–Ottawa scale

		Selection				*Comparability*	*Outcome*			*Total*	*Risk of bias of included studies*
Study	*Year*	*S1*	*S2*	*S3*	*S4*	*C1*	*O1*	*O2*	*O3*	*Scores*	
Gaziano *et al.*	1995	1	1	1		1	1		1	6	High
Joshipura *et al.*	1999	1	1	1	1	2	1	1	1	9	Low
Joshipura *et al.*	2001	1	1	1	1	2		1	1	8	Moderate
Johnsen *et al.*	2003	1	1	1	1	2	1		1	8	Moderate
Sauvaget *et al.*	2003	1	1		1	2	1	1	1	8	Moderate
Hung *et al.*	2004	1	1	1	1	2	1	1	1	9	Low
Takachi *et al.*	2007	1	1	1		2	1		1	7	Moderate
Joshipura *et al.*	2008	1	1	1	1	2		1	1	8	Moderate
Bendinelli *et al.*	2010	1	1	1	1	2	1	1	1	9	Low
Oude *Griep et al.*	2011A	1	1	1	1	2	1	1	1	9	Low
Oude Griep *et al.*	2011B	1	1	1	1	2	1	1	1	9	Low
Larsson *et al.*	2013	1	1	1	1	2	1	1	1	9	Low
Bhupathiraju *et al.*	2013	1	1	1	1	2	1	1	1	9	Low
Rautiainen *et al.*	2014	1	1	1	1	2	1	1	1	9	Low
Buil-Cosiales *et al.*	2016	1	1	1	1	2	1	1	1	9	Low
Wang *et al.*	2016	1	1	1		2	1	1	1	8	Moderate
Blekkenhorst *et al.*	2017	1	1	1	1	2	1	1	1	9	Low

Risk of bias of included studies: high risk of bias: ≤ 6; moderate risk of bias: 7–8; low risk of bias: 9 and empty cells indicate a score of 0.
S1 – representativeness of the exposed cohort; S2 – selection of the non-exposed cohort; S3 – ascertainment of exposure; S4 – demonstration that outcome of interest was absent at the start of the study; C1 – comparability of the cohort based on the design or analysis; O1 – assessment of outcome; O2 – was follow up long enough for outcomes to occur?; O3 – adequacy of follow up of cohorts.

Studies on this subject (Table 1) were published over 12 years (1995–2017). Most reports assessed GLV intakes using the food-frequency questionnaire, but limited studies[42,45,50] adjusted for total energy intakes (and other dietary confounding factors) in

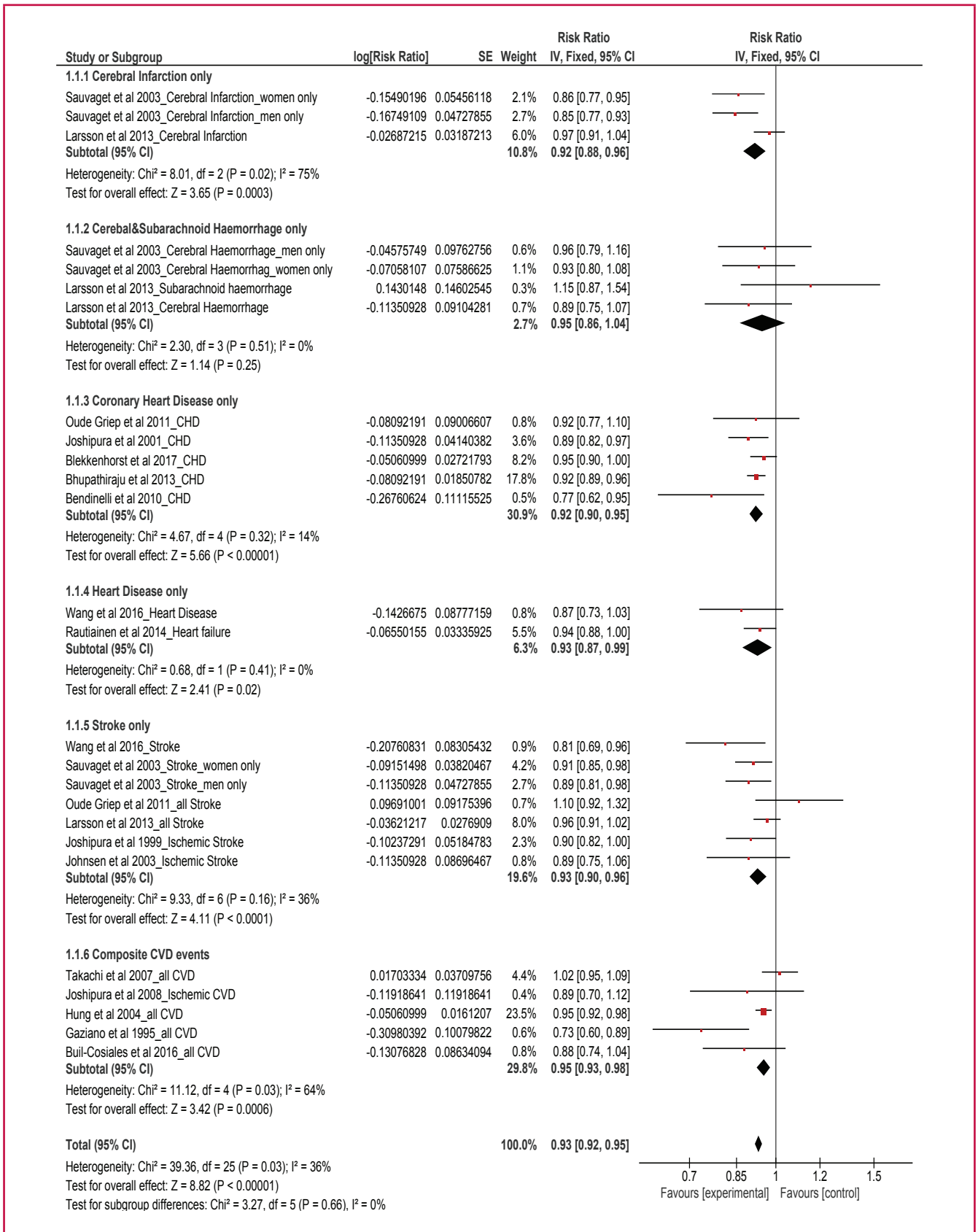

Fig. 2. Relative risk, 95% CI and *p*-value of incidence of all CVD events in the meta-analysis.

the multivariate analysis of GLV and CVD outcomes.

More than half of the studies included in this report presented a low risk of bias (Table 2). In all, methodological assessment of included reports revealed no evidence of high risk of bias in most studies included in the meta-analysis.

Overall, higher intake of GLV (Fig. 2) was associated with reduced incidence of all CVD events by 7% (RR: 0.93; 95% CI: 0.92–0.95; $p < 0.00001$). Similarly, higher GLV intake was inversely related to the incidence of cerebral infarction (RR: 0.92; 95% CI: 0.88–0.96; $p = 0.0003$), CHD (RR: 0.92; 95% CI: 0.90–0.95; $p < 0.00001$), heart disease (RR: 0.93; 95% CI: 0.87–0.99; $p = 0.02$) and stroke (RR: 0.93; 95% CI: 0.90–0.96; $p < 0.0001$). The result remained unchanged after stratifying the studies by gender of respondents (Fig. 3A); men (RR: 0.92; 95% CI: 0.88–0.96; $p < 0.0001$) and women (RR: 0.92; 95% CI: 0.89–0.95; $p < 0.00001$).

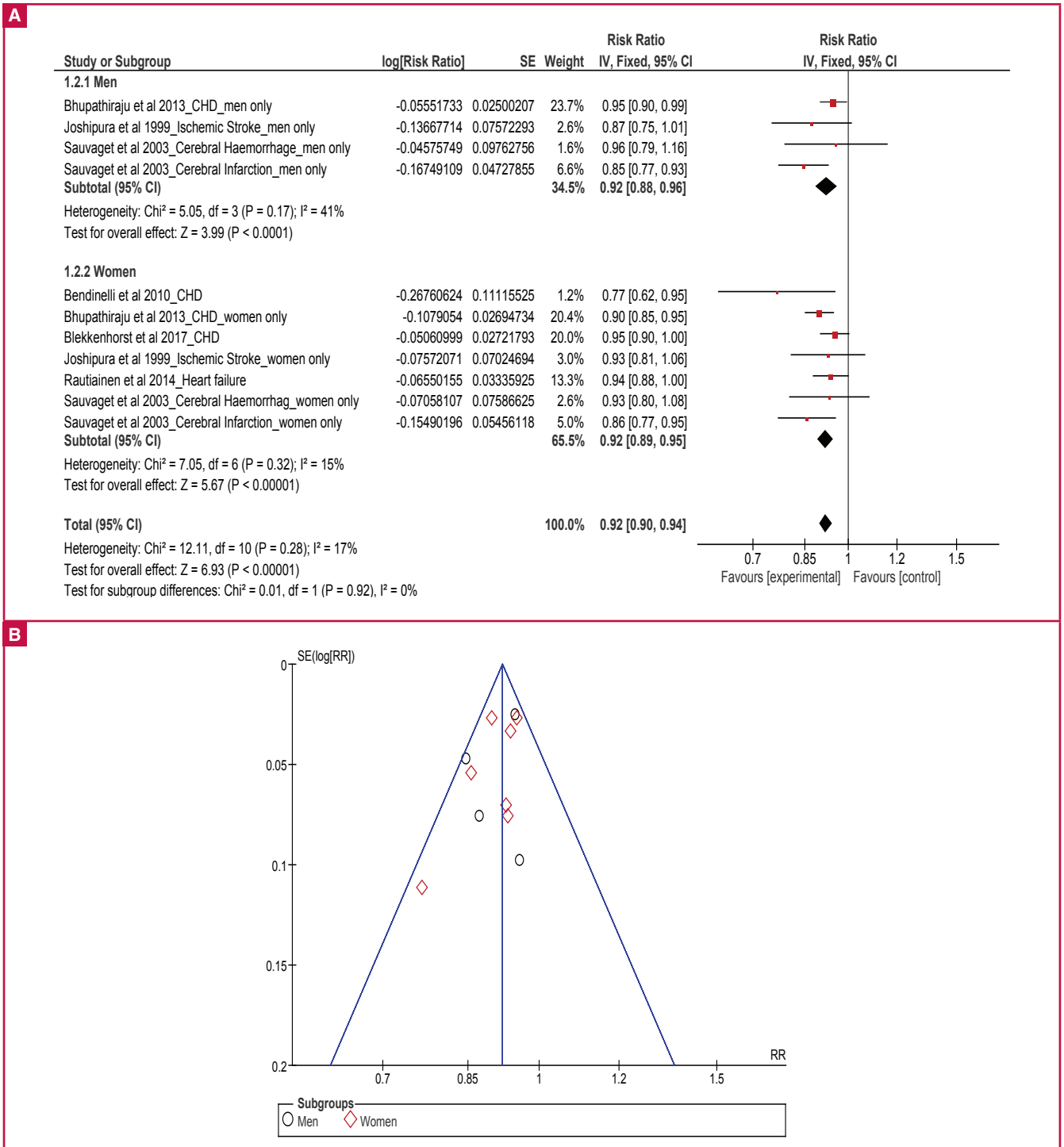

Fig. 3. Relative risk, 95% CI and *p*-value of incidence (A) and funnel plot (B) of all CVD events stratified by gender in the meta-analysis.

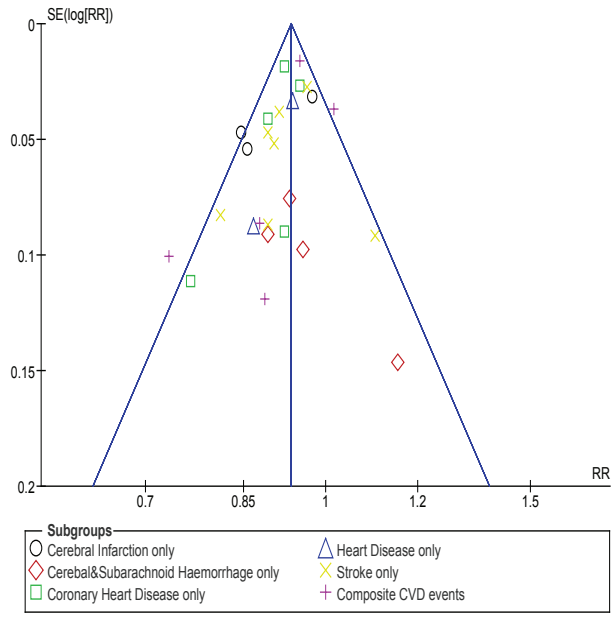

Fig. 4. Funnel plots assessing publication bias in the meta-analysis.

Table 3. Sensitivity analysis of pooled RR stratified by categories of CVD events in the meta-analysis

Studies in the meta-analysis	I^2 *(%)*	*Pooled RR (95% CI)*	p-*value*
All studies	36	0.93 (0.92–0.95)	< 0.00001
Cerebral infarction only	28	0.94 (0.92–0.95)	< 0.00001
Cerebal and subarachnoid haemorrhage only	43	0.93 (0.92–0.95)	< 0.00001
Coronary heart disease only	41	0.94 (0.92–0.96)	< 0.00001
Heart disease only	40	0.93 (0.92–0.95)	< 0.00001
Stroke only	40	0.93 (0.92–0.95)	< 0.00001
Composite CVD events	22	0.93 (0.91–0.94)	< 0.00001

Statistical heterogeneity (Fig. 1) was low for studies on heart disease only (I^2 = 0%), CHD only (I^2 = 14%), and stroke only (I^2 = 36%) but not among studies on cerebral infarction only (I^2 = 75%).

Funnel plots (Figs 3B, 4) suggested no evidence of publication bias and no sole study exerted a significant effect on the sensitivity of the overall findings of the meta-analysis (Tables 3, 4).

Table 4. Sensitivity analysis of pooled RR of all cohort studies included in the meta-analysis

Studies in the meta-analysis	I^2 *(%)*	*Pooled RR (95% CI)*	p-*value*
Cerebral infarction only			
All studies	75	0.92 (0.88–0.96)	0.0003
Larsson *et al.* 2013_Cerebral infarction	0	0.85 (0.79–0.91)	< 0.00001
Sauvaget *et al.* 2003_Cerebral infarction_men only	76	0.94 (0.89–0.99)	0.03
Sauvaget *et al.* 2003_Cerebral infarction_women only	84	0.93 (0.88–0.98)	0.007
Cerebal and subarachnoid haemorrhage only			
All studies	0	0.95 (0.86–1.04)	0.25
Larsson *et al.* 2013_Cerebral haemorrhage	0	0.97 (0.87–1.08)	0.57
Larsson *et al.* 2013_Subarachnoid haemorrhage	0	0.93 (0.84–1.02)	0.12
Sauvaget *et al.* 2003_Cerebral haemorrhage_women only	0	0.96 (0.85–1.08)	0.48
Sauvaget *et al.* 2003_Cerebral haemorrhage_men only	13	0.95 (0.85–1.05)	0.30
Coronary heart disease only			
All studies	14	0.92 (0.90–0.95)	< 0.00001
Bendinelli *et al.* 2010_CHD	0	0.93 (0.90–0.95)	< 0.00001
Bhupathiraju *et al.* 2013_CHD	36	0.93 (0.89–0.97)	0.0003
Blekkenhorst *et al.* 2017_CHD	4	0.91 (0.88–0.94)	< 0.00001
Joshipura *et al.* 2001_CHD	23	0.93 (0.90–0.96)	< 0.00001
Oude Griep *et al.* 2011_CHD	36	0.92 (0.90–0.95)	< 0.00001
Heart disease only			
All studies	0	0.93 (0.87–0.99)	0.02
Rautiainen *et al.* 2014_Heart failure	–	0.87 (0.73–1.03)	0.10
Wang *et al.* 2016_Heart disease	–	0.94 (0.88–1.00)	0.05
Stroke only			
All studies	36	0.93 (0.90–0.96)	< 0.0001
Johnsen *et al.* 2003_Ischemic stroke	45	0.93 (0.90–0.97)	< 0.0001
Joshipura *et al.* 1999_Ischemic stroke	44	0.93 (0.90–0.97)	0.0003
Larsson *et al.* 2013_all stroke	22	0.91 (0.87–0.95)	< 0.0001
Oude Griep *et al.* 2011_all stroke	14	0.92 (0.89–0.96)	< 0.0001
Sauvaget *et al.* 2003_Stroke_men only	41	0.94 (0.90–0.97)	0.0005
Sauvaget *et al.* 2003_Stroke_women only	45	0.93 (0.90–0.97)	0.0007
Wang *et al.* 2016_Stroke	24	0.94 (0.90–0.97)	0.0003
Composite CVD events			
All studies	64	0.95 (0.93–0.98)	0.0006
Buil-Cosiales *et al.* 2016_all CVD	75	0.95 (0.93–0.98)	0.001
Gaziano *et al.* 1995_all CVD	30	0.96 (0.93–0.98)	0.003
Hung *et al.* 2004_all CVD	73	0.96 (0.90–1.02)	0.17
Joshipura *et al.* 2008_Ischemic CVD	72	0.95 (0.93–0.98)	0.0009
Takachi *et al.* 2007_all CVD	59	0.94 (0.91–0.97)	< 0.0001

Discussion

In this study, higher intake of GLV was linked to reduced incidence of all CVD events by 7% and, in particular, it was inversely related to the incidence of cerebral infarction, CHD, heart disease and stroke. These findings may suggest a potential role of GLV intake as a primary-prevention strategy in the management of CVD.

Similar to our findings, the largest study on stroke among Africans [the Stroke Investigative Research and Educational Network (SIREN) study] reported a strong protective dose–response association such that daily consumption of GLV was more protective against stroke [odds ratio (OR): 0.27; 95% CI: 0.19–0.38] than weekly consumption (OR: 0.70; 95% CI: 0.52–0.95), compared to no consumption.[52] Earlier systematic reviews and meta-analyses were broadly focused and generally combined fruit and vegetables in investigating the effect of these nutritional modalities on incident CVD events.[11,19,20,22,53-58] The uniqueness of our study is therefore in the deconstruction of the specific contribution of GLV on CVD. Also, our approach offered vital insights into the potential roles of GLV in the occurrence of CVD subtypes.

Although the exact mechanism of the protective effect of GLV is not well understood, some constituents of GLV are likely to confer small-to-moderate but clinically important protection against CVD.[25] For example, Vitamin B_9, micronutrients and other constituents of GLV are known to promote optimal health and

protect against several diseases.[29,59] The fibre component of GLV is also known for its cholesterol-lowering effects.[60] Similarly, folic acid (a constituent of GLV) intake is inversely associated with homocysteinaemia,[61,62] a known risk factor for atherosclerosis and ischaemic stroke.[63-65] Furthermore, micronutrients in GLV may promote cardiovascular integrity, haemostasis (Vitamin K content), neuronal transmission (calcium content), antioxidant activity (vitamins C and E content)[32,66] and vasodilatory effects (nitrates content).[67,68]

There are existing gaps in the literature on the effect of GLV on CVD outcomes not covered by the present systematic review and meta-analysis. For example, the mode of preparation and preservation of GLV on CVD outcomes remains unclear. Similarly, the underlying molecular mechanisms mediating the protective effect of GLV remains incomplete. These gaps in our understanding of the relationship between GLV and CVD could be the basis of future cohort studies and clinical trials.

Limitations, strengths and recommendations

GLV are not consumed singly in diets. Similarly, higher GLV consumption in the presence of exposure to traditional risk factors of CVD (such as smoking, alcohol intake, low physical activity) does not imply less CVD risk. Our study considered populations exposed to higher GLV intakes in their overall diet only, independent of the magnitude of consumption of other food items.

This systematic review and meta-analysis has other limitations. First, this meta-analysis did not investigate the relationship between GLV and CVD outcomes according to ethnic background and country of study due to the limited number of studies on the subject. Most studies were from the United States. There were limited studies from populations of African and Asian ancestry. This hindered us from performing subgroup analyses by region and ethnicity as indicated in the study protocol.

Second, there were methodological differences in the estimation of GLV intake among studies included in this systematic review and meta-analysis. However, these differences are likely insignificant given the consistent direction and strength of the relationship in our reported pooled-effect estimate after stratifying the meta-analysis across several subgroups. However, it is necessary to establish models that can uniformly quantitate GLV consumption across different populations.

Third, our search for grey literature was limited to informal requests for unpublished data and reports on the effect of GLV on CVD from local specialists in human nutritional research. This strategy did not result in the retrieval of additional primary data suitable for our meta-analysis objectives.

A key strength of our study is that it may be the first to summarise data on the association between GLV intake and not only incident CVD events in general but also subtypes of these outcomes.

Conclusion

Our meta-analysis demonstrated that a higher intake of GLV was associated with a lower incidence of CVD events, independent of subtypes of CVD manifestation. Promoting the consumption of GLV may be useful for the management and prevention of CVD. Also, dietary strategies that incorporate GLV consumption may be encouraged and promoted. Further studies are necessary to determine the underlying mechanism(s) and the significance of duration of exposure on the magnitude of the effect of GLV on CVD events. In particular, a future multicentre cohort study with uniform quantification of GLV consumption and duration between exposure and CVD events would be desirable to confirm these findings.

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