Review Article

# Dietary intakes of green leafy vegetables and incidence of cardiovascular diseases

Akin Ojagbemi, Akinkunmi Paul Okekunle, Paul Olowoyo, Onoja Matthew Akpa, Rufus Akinyemi, Bruce Ovbiagele, Mayowa Owolabi

## 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]

**Department of Psychiatry, College of Medicine, University of Ibadan, Ibadan, Nigeria**
Akin Ojagbemi, MB BS, MSc, PhD

**Department of Medicine, College of Medicine, University of Ibadan; University College Hospital, Ibadan, Nigeria**
Akinkunmi Paul Okekunle, PhD, akinokekunle@gmail.com
Mayowa Owolabi, MB BS, MSc, DrM, FAAN, FANA, FRCP, FAcadMedS, FAS, mayowaowolabi@yahoo.com

**Department of Epidemiology and Medical Statistics, College of Medicine, University of Ibadan, Ibadan, Nigeria**
Akinkunmi Paul Okekunle, PhD
Onoja Matthew Akpa, BSc, MSc, PhD

**Epidemiology Laboratory, Department of Food and Nutrition, College of Human Ecology, Seoul National University, Seoul, Korea**
Akinkunmi Paul Okekunle, PhD

**College of Medicine and Health Sciences, Afe Babalola University; Department of Medicine, Federal Teaching Hospital, Ado-Ekiti, Nigeria**
Paul Olowoyo, MB BS, FWACP

**Centre for Genomic and Precision Medicine, College of Medicine, University of Ibadan, Ibadan, Nigeria**
Onoja Matthew Akpa, BSc, MSc, PhD
Rufus Akinyemi, MB BS, MSc, PhD, MWACP, FMCP
Mayowa Owolabi, MB BS, MSc, DrM, FAAN, FANA, FRCP, FAcadMedS, FAS, mayowaowolabi@yahoo.com

**Institute of Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria**
Rufus Akinyemi, MB BS, MSc, PhD, MWACP, FMCP

**Weill Institute for Neurosciences; School of Medicine, University of California, San Francisco, California, USA**
Bruce Ovbiagele, MB BS, MSc, MAS, MBA, FAAN

**Department of Medicine, College of Medicine, University of Ibadan; University College Hospital; Blossom Specialist Medical Centre, Ibadan, Nigeria**
Mayowa Owolabi, MB BS, MSc, DrM, FAAN, FANA, FRCP, FAcadMedS, FAS, mayowaowolabi@yahoo.com

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

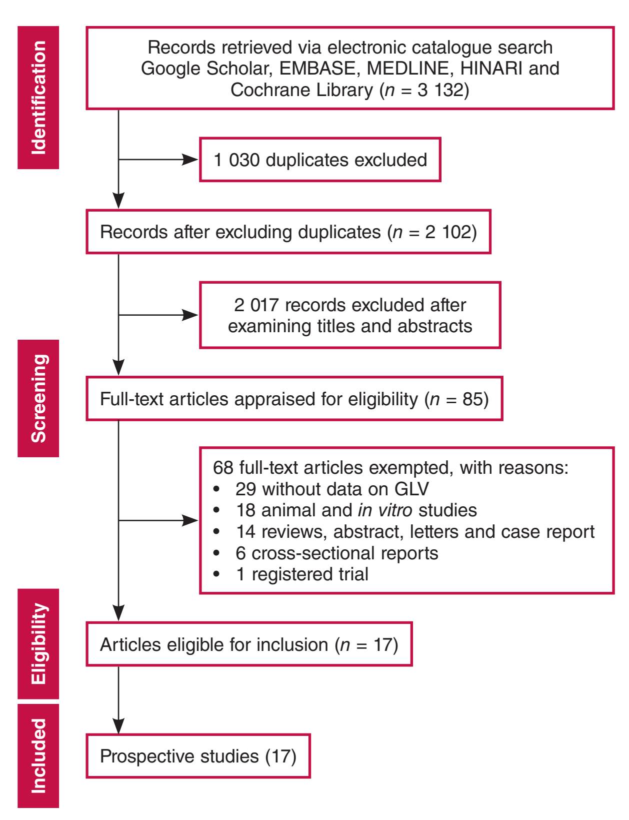


**Fig. 1.** PRISMA flowchart describing selection of the studies for the meta-analysis

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

| | *Study characteristics* | | | *Baselineloutcomes 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 | [C]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 | [C]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; [C]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.

consumption (as reference) for the incidence of CVD events 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

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

| | | *Selection* | | | | *Comparability* | *Outcome* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Study* | *Year* | *S1* | *S2* | *S3* | *S4* | *C1* | *O1* | *O2* | *O3* | *Total Scores* | *Risk of bias of included studies* |
| 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.

six reports on stroke[46-51]) were included in the meta-analysis.

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

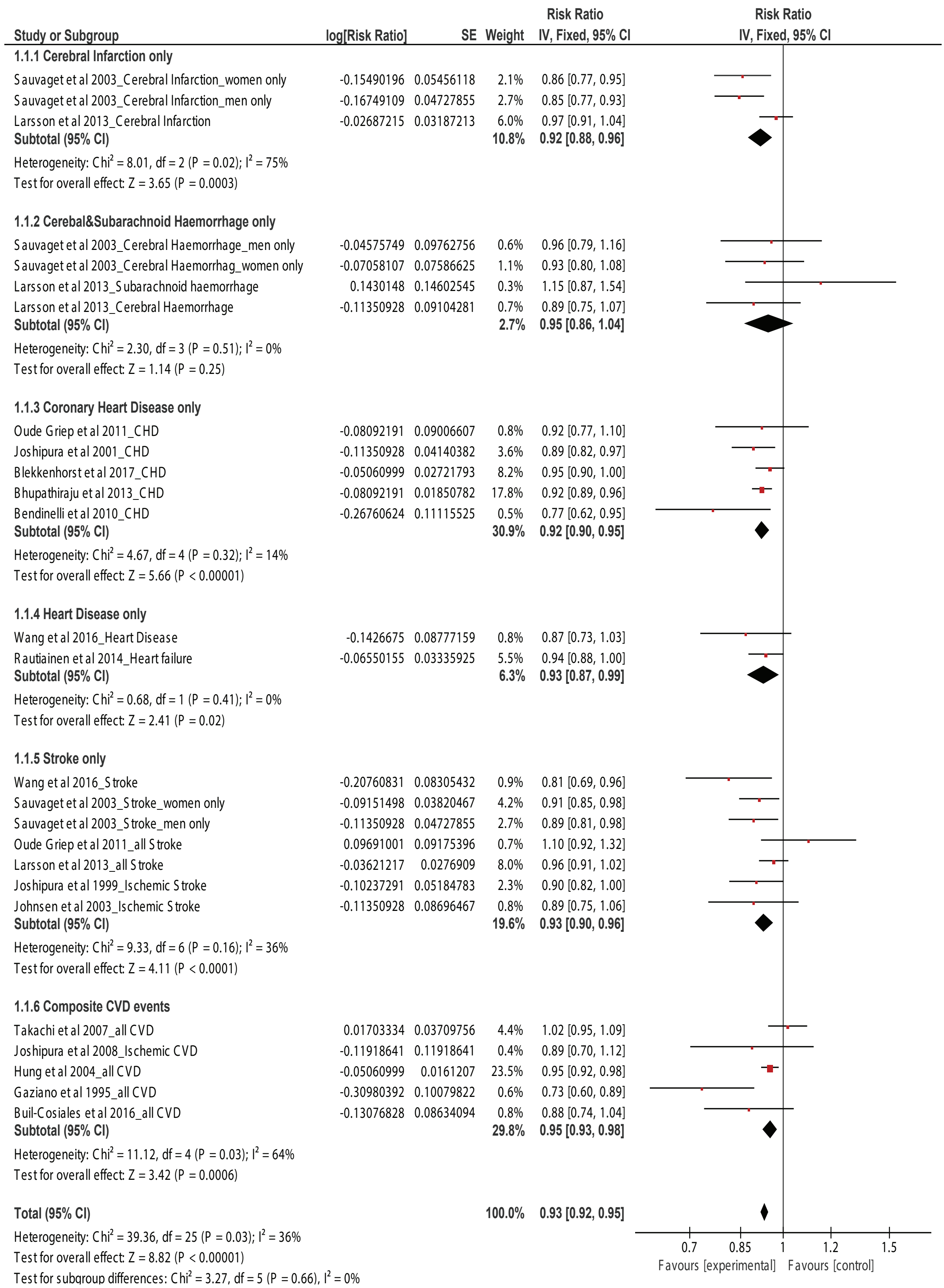


**Fig. 2.** Relative risk, 95% CI and $p$-value of incidence of all CVD events in the meta-analysis.

total energy intakes (and other dietary confounding factors) in 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:

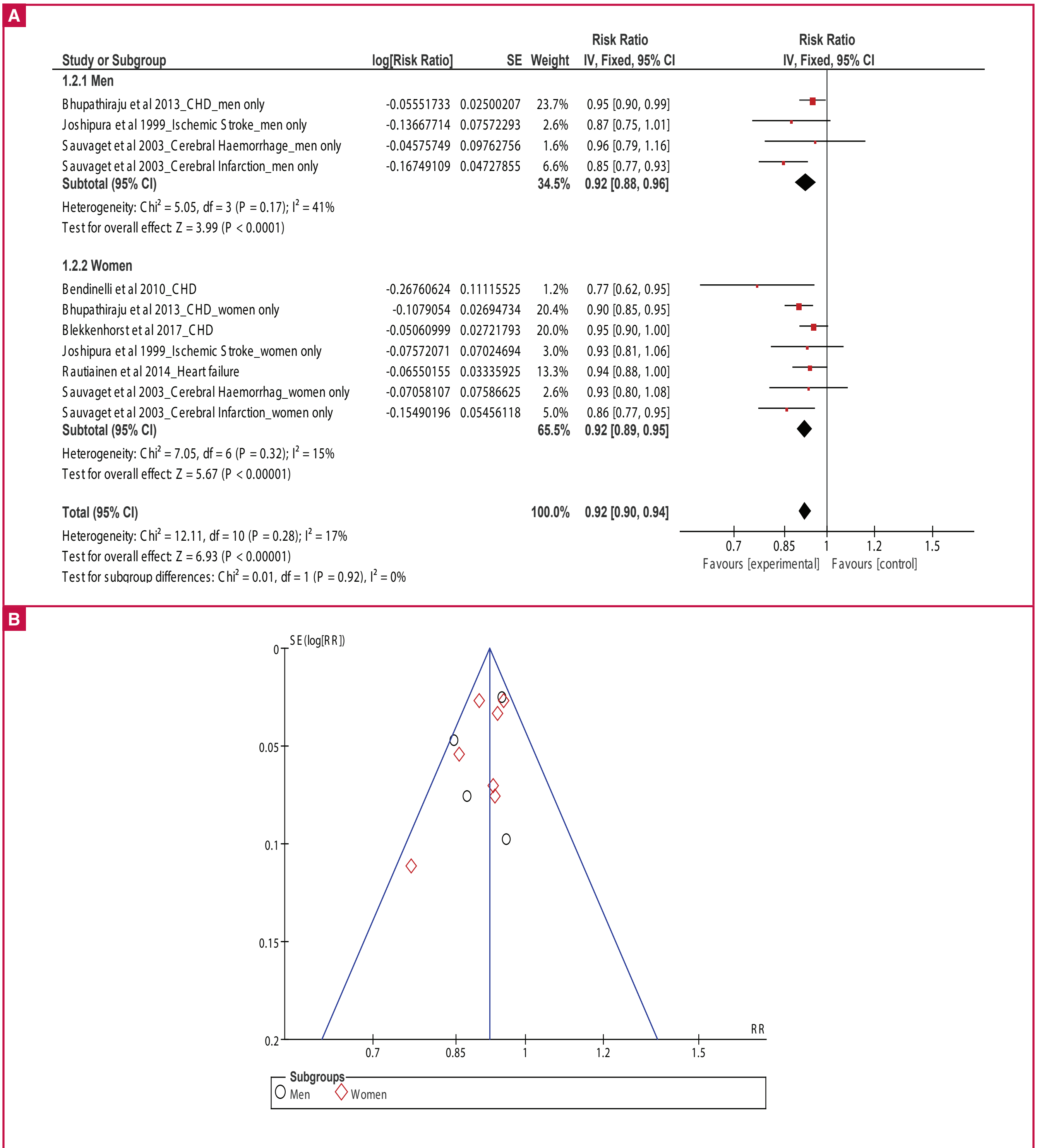


**Fig. 3.** Relative risk, 95% CI and *p*-value of incidence (A) and funnel plot (B) of all CVD events stratified by gender in the meta-analysis.

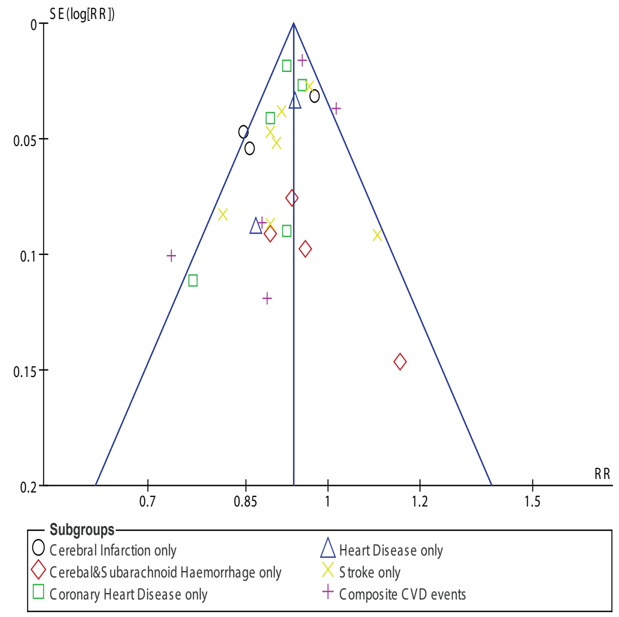


**Fig. 4.** Funnel plots assessing publication bias in the meta-analysis.

**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 |

0.89–0.95; $p < 0.00001$).

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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## References

1. WHO. Cardiovascular diseases (CVDs): WHO; 2017 [cited 2020 03.08.2020]. Available from: https://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).
2. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, *et al.* Heart disease and stroke statistics – 2013 update: a report from the American Heart Association. *Circulation* 2013; **127**(1): e6–e245.
3. Heidenreich PA, Trogdon JG, Khavjou OA, Butler J, Dracup K, Ezekowitz MD, *et al*. Forecasting the future of cardiovascular disease in the United States. *Circulation* 2011; **123**(8): 933–944.
4. Benjamin EJ, Virani SS, Callaway CW, Chamberlain AM, Chang AR, Cheng S, *et al*. Heart disease and stroke statistics – 2018 update: a report from the American Heart Association. *Circulation* 2018; **137**(12): e67–e492.
5. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, *et al.* Heart disease and stroke statistics – 2015 update: a report from the American Heart Association. *Circulation* 2015; **131**(4): e29–322.
6. Virani SS, Alonso A, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, *et al.* Heart disease and stroke statistics – 2020 update: a report from the American Heart Association. *Circulation* 2020; **141**(9): e139–e596.
7. Krishnamurthi RV, Feigin VL, Forouzanfar MH, Mensah GA, Connor M, Bennett DA, *et al*. Global and regional burden of first-ever ischaemic and haemorrhagic stroke during 1990–2010: findings from the Global Burden of Disease Study 2010. *Lancet Glob Health* 2013; **1**(5): e259–e281.
8. Leong DP, Joseph PG, McKee M, Anand SS, Teo KK, Schwalm J-D, *et al*. Reducing the global burden of cardiovascular disease, Part 2. *Circ Res* 2017; **121**(6): 695–710.
9. Joseph P, Leong D, McKee M, Anand SS, Schwalm J-D, Teo K, et al. Reducing the global burden of cardiovascular disease, Part 1. *Circ Res* 2017; **121**(6): 677–694.
10. Buil-Cosiales P, Toledo E, Salas-Salvadó J, Zazpe I, Farràs M, Basterra-Gortari FJ, *et al.* Association between dietary fibre intake and fruit, vegetable or whole-grain consumption and the risk of CVD: results from the PREvención con DIeta MEDiterránea (PREDIMED) trial. *Br J Nutr* 2016; **116**(3): 534–546.
11. Rees K, Takeda A, Martin N, Ellis L, Wijesekara D, Vepa A, *et al.* Mediterranean-style diet for the primary and secondary prevention of cardiovascular disease. *Cochrane Database Syst Rev* 2019; 3.

12. Hartley L, Igbinedion E, Holmes J, Flowers N, Thorogood M, Clarke A, *et al*. Increased consumption of fruit and vegetables for the primary prevention of cardiovascular diseases. *Cochrane Database Syst Rev* 2013; 6.
13. Jenkins DJA, Spence JD, Giovannucci EL, Kim YI, Josse R, Vieth R, *et al.* Supplemental vitamins and minerals for CVD prevention and treatment. *J Am Coll Cardiol* 2018; **71**(22): 2570–2584.
14. Kaluza J, Wolk A, Larsson SC. Red meat consumption and risk of stroke: a meta-analysis of prospective studies. *Stroke* 2012; **43**(10): 2556–2560.
15. Yang C, Pan L, Sun C, Xi Y, Wang L, Li D. Red meat consumption and the risk of stroke: a dose–response meta-analysis of prospective cohort studies. *J Stroke Cerebrovasc Dis* 2016; **25**(5): 1177–1186.
16. Strazzullo P, D'Elia L, Kandala NB, Cappuccio FP. Salt intake, stroke, and cardiovascular disease: meta-analysis of prospective studies. *Br Med J* 2009; **339**: 4567.
17. Renaud SC. Diet and stroke. *J Nutr Health Aging* 2001; **5**(3): 167–172.
18. Spence JD. Diet for stroke prevention. *Stroke Vasc Neurol* 2018; **3**(2): 44–50.
19. Deng C, Lu Q, Gong B, Li L, Chang L, Fu L, *et al*. Stroke and food groups: an overview of systematic reviews and meta-analyses. *Public Health Nutr* 2017; **21**(4): 766–776.
20. Mytton OT, Nnoaham K, Eyles H, Scarborough P, Ni Mhurchu C. Systematic review and meta-analysis of the effect of increased vegetable and fruit consumption on body weight and energy intake. *BMC Public Health* 2014; **14**(1): 886.
21. Pollock RL. The effect of green leafy and cruciferous vegetable intake on the incidence of cardiovascular disease: A meta-analysis. *JRSM Cardiovasc Dis* 2016; **5**: 2048004016661435.
22. Yip CSC, Chan W, Fielding R. The associations of fruit and vegetable intakes with burden of diseases: a systematic review of meta-analyses. *J Acad Nutr Diet* 2019; **119**(3): 464–481.
23. Aune D, Giovannucci E, Boffetta P, Fadnes LT, Keum N, Norat T, *et al.* Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality – a systematic review and dose-response meta-analysis of prospective studies. *Int J Epidemiol* 2017; **46**(3): 1029–1056.
24. Kwok CS, Gulati M, Michos ED, Potts J, Wu P, Watson L, *et al*. Dietary components and risk of cardiovascular disease and all-cause mortality: a review of evidence from meta-analyses. *Eur J Prevent Cardiol* 2019; **26**(13): 1415–1429.
25. Gaziano JM, Manson JE, Branch LG, Colditz GA, Willett WC, Buring JE. A prospective study of consumption of carotenoids in fruits and vegetables and decreased cardiovascular mortality in the elderly. *Ann Epidemiol* 1995; **5**(4): 255–260.
26. Hung H-C, Joshipura KJ, Jiang R, Hu FB, Hunter D, Smith-Warner SA, *et al.* Fruit and vegetable intake and risk of major chronic disease. *J Nat Cancer Instit* 2004; **96**(21): 1577–1584.
27. Takachi R, Inoue M, Ishihara J, Kurahashi N, Iwasaki M, Sasazuki S, *et al*. Fruit and vegetable intake and risk of total cancer and cardiovascular disease: Japan public health center-based prospective study. *Am J Epidemiol* 2007; **167**(1): 59–70.
28. Oude Griep LM, Monique Verschuren WM, Kromhout D, Ocké MC, Geleijnse JM. Colours of fruit and vegetables and 10-year incidence of CHD. *Br J Nutr* 2011; **106**(10): 1562–1569.
29. Moyo SM, Serem JC, Bester MJ, Mavumengwana V, Kayitesi E. African Green leafy vegetables: health benefits beyond nutrition. *Food Rev Int* 2020: 1–18.
30. Gertsch J. The metabolic plant feedback hypothesis: how plant secondary metabolites nonspecifically impact human health. *Planta Med* 2016; **82**(11/12): 920–929.
31. Fang X, Liang C, Li M, Montgomery S, Fall K, Aaseth J, *et al*. Dose–response relationship between dietary magnesium intake and cardiovascular mortality: A systematic review and dose-based meta-regression analysis of prospective studies. *J Trace Elem Med Biol* 2016; **38**: 64–73.
32. Jiménez-Aguilar DM, Grusak MA. Minerals, vitamin C, phenolics, flavonoids and antioxidant activity of Amaranthus leafy vegetables. *J Food Compost Anal* 2017; **58**: 33–39.
33. Ejoh SI, Wireko-Manu FD, Page D, Mgc Renard C. Traditional green leafy vegetables as underutilised sources of micronutrients in a rural farming community in south-west Nigeria II: consumption pattern and potential contribution to micronutrient requirements. *S Afr J Clin Nutr* 2019: 1–6.
34. Ejoh SI, Wireko-Manu FD, Page D, Renard CMGC. Traditional green leafy vegetables as underutilised sources of micronutrients in a rural farming community in south-west Nigeria I: estimation of vitamin C, carotenoids and mineral contents. *S Afr J Clin Nutr* 2019: 1–6.
35. Ascherio A, Rimm EB, Hernán MA, Giovannucci EL, Kawachi I, Stampfer MJ, *et al*. Intake of potassium, magnesium, calcium, and fiber and risk of stroke among US men. *Circulation* 1998; **98**(12): 1198–1204.
36. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, *et al*. The Newcastle–Ottawa scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses Canada: Ottawa Hospital Research Institute. Available from http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp; 2014 [updated 2014. Available from: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.
37. Higgins JPT, Green S, editors. Cochrane Handbook for Systematic Reviews of Interventions: The Cochrane Collaboration. Available from www.cochrane-handbook.org. 2011.
38. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; **21**(11): 1539–1558.
39. Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration. 2014.
40. Joshipura KJ, Hung H-C, Li TY, Hu FB, Rimm EB, Stampfer MJ, *et al*. Intakes of fruits, vegetables and carbohydrate and the risk of CVD. *Public Health Nutr* 2009; **12**(1): 115–121.
41. Bendinelli B, Masala G, Saieva C, Salvini S, Calonico C, Sacerdote C, *et al*. Fruit, vegetables, and olive oil and risk of coronary heart disease in Italian women: the EPICOR Study. *Am J Clin Nutr* 2011; **93**(2): 275–283.
42. Bhupathiraju SN, Wedick NM, Pan A, Manson JE, Rexrode KM, Willett WC, *et al*. Quantity and variety in fruit and vegetable intake and risk of coronary heart disease. *Am J Clin Nutr* 2013; **98**(6): 1514–1523.
43. Joshipura KJ, Hu FB, Manson JE, Stampfer MJ, Rimm EB, Speizer FE, *et al.* The effect of fruit and vegetable intake on risk for coronary heart disease. *Ann Intern Med* 2001; **134**(12): 1106–1114.
44. Blekkenhorst LC, Bondonno CP, Lewis JR, Devine A, Zhu K, Lim WH, *et al.* Cruciferous and allium vegetable intakes are inversely associated with atherosclerotic vascular disease deaths in older adult women. *J Am Heart Assoc* 2017; **6**(10): e006558.
45. Rautiainen S, Levitan EB, Mittleman MA, Wolk A. Fruit and vegetable intake and rate of heart failure: a population-based prospective cohort of women. *Eur J Heart Fail* 2015; **17**(1): 20–26.
46. Joshipura KJ, Ascherio A, Manson JE, Stampfer MJ, Rimm EB, Speizer FE, *et al.* Fruit and vegetable intake in relation to risk of ischemic stroke. *J Am Med Assoc* 1999; **282**(13): 1233–1239.
47. Johnsen SP, Overvad K, Stripp C, Tjonneland A, Husted SE, Sorensen HT. Intake of fruit and vegetables and the risk of ischemic stroke in a cohort of Danish men and women. *Am J Clin Nutr* 2003; **78**(1): 57–64.
48. Sauvaget C, Nagano J, Allen N, Kodama K. Vegetable and fruit intake and stroke mortality in the Hiroshima/Nagasaki Life Span Study. *Stroke* 2003; **34**(10): 2355–2360.
49. Griep LMO, Verschuren WMM, Kromhout D, Ocké MC, Geleijnse JM. Colors of fruit and vegetables and 10-year incidence of stroke. *Stroke* 2011;

**42**(11): 3190–3195.

50. Larsson SC, Virtamo J, Wolk A. Total and specific fruit and vegetable consumption and risk of stroke: A prospective study. *Atherosclerosis* 2013; **227**(1): 147–152.
51. Wang J-B, Fan J-H, Dawsey SM, Sinha R, Freedman ND, Taylor PR, *et al*. Dietary components and risk of total, cancer and cardiovascular disease mortality in the Linxian Nutrition Intervention Trials cohort in China. *Sci Rep* 2016; **6**: 22619.
52. Owolabi MO, Sarfo F, Akinyemi R, Gebregziabher M, Akpa O, Akpalu A, *et al*. Dominant modifiable risk factors for stroke in Ghana and Nigeria (SIREN): a case–control study. *Lancet Glob Health* 2018; **6**(4): e436–e46.
53. Angelino D, Godos J, Ghelfi F, Tieri M, Titta L, Lafranconi A, *et al*. Fruit and vegetable consumption and health outcomes: an umbrella review of observational studies. *Int J Food Sci Nutr* 2019; **70**(6): 652–667.
54. Bazzano LA, Serdula MK, Liu S. Dietary intake of fruits and vegetables and risk of cardiovascular disease. *Curr Atheroscler Rep* 2003; **5**(6): 492–499.
55. Chiavaroli L, Viguiliouk E, Nishi SK, Blanco Mejia S, Rahelić D, Kahleová H, *et al.* DASH dietary pattern and cardiometabolic outcomes: an umbrella review of systematic reviews and meta-analyses. *Nutrients* 2019; **11**(2): 338.
56. Danaei G, Ding EL, Mozaffarian D, Taylor B, Rehm J, Murray CJL, *et al.* The preventable causes of death in the United States: comparative risk assessment of dietary, lifestyle, and metabolic risk factors. *PLoS Med* 2009; **6**(4): e1000058.
57. Tada N, Maruyama C, Koba S, Tanaka H, Birou S, Teramoto T, *et al.* Japanese dietary lifestyle and cardiovascular disease. *J Atheroscler Thromb* 2011; **18**(9): 723–734.
58. Rees K, Hartley L, Flowers N, Clarke A, Hooper L, Thorogood M, *et al.* 'Mediterranean' dietary pattern for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev* 2013(8): CD009825.
59. Roberts JL, Moreau R. Functional properties of spinach (*Spinacia oleracea* L.) phytochemicals and bioactives. *Food Funct* 2016; **7**(8): 3337–3353.
60. Brown L, Rosner B, Willett WW, Sacks FM. Cholesterol-lowering effects of dietary fiber: a meta-analysis. *Am J Clin Nutr* 1999; **69**(1): 30–42.
61. He K, Merchant A, Rimm EB, Rosner BA, Stampfer MJ, Willett WC, *et al.* Folate, Vitamin $B_6$ and $B_{12}$ intakes in relation to risk of stroke among men. *Stroke* 2004; **35**(1): 169–174.
62. Verly-Jr E, Steluti J, Fisberg RM, Marchioni DML. A quantile regression approach can reveal the effect of fruit and vegetable consumption on plasma homocysteine levels. *PLoS One* 2014; **9**(11): e111619.
63. Han L, Wu Q, Wang C, Hao Y, Zhao J, Zhang L, *et al*. Homocysteine, ischemic stroke, and coronary heart disease in hypertensive patients. *Stroke* 2015; **46**(7): 1777–1786.
64. Rita C, Nagaraja D, Shankar SK. Homocysteine and cerebral stroke in developing countries. *Curr Med Chem* 2007; **14**(22): 2393–2401.
65. Modi M, Prabhakar S, Majumdar S, Khullar M, Lal V, Das C. Hyperhomocysteinemia as a risk factor for ischemic stroke: An Indian scenario. *Neurol India* 2005; **53**(3): 297–301.
66. Oboh G, Raddatz H, Henle T. Antioxidant properties of polar and non-polar extracts of some tropical green leafy vegetables. *J Sci Food Agric* 2008; **88**(14): 2486–2492.
67. Lidder S, Webb AJ. Vascular effects of dietary nitrate (as found in green leafy vegetables and beetroot) via the nitrate–nitrite–nitric oxide pathway. *Br J Clin Pharmacol* 2013; **75**(3): 677–696.
68. Ashworth A, Mitchell K, Blackwell JR, Vanhatalo A, Jones AM. High-nitrate vegetable diet increases plasma nitrate and nitrite concentrations and reduces blood pressure in healthy women. *Public Health Nutr* 2015; **18**(14): 2669–2678.

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'We've long known that a difference in blood pressure between the two arms is linked to poorer health outcomes. The large numbers involved in the INTERPRESS-IPD study help us to understand this in more detail. It tells us that the higher the difference in blood pressure between arms, the greater the cardiovascular risk, so it really is critical to measure both arms to establish which patients may be at significantly increased risk. Patients who require a blood pressure check should now expect that it's checked in both arms, at least once.'

Blood pressure rises and falls in a cycle with each pulse. It is measured in units of millimetres of mercury (mmHg), and the reading is always given as two numbers: the upper (systolic) reading represents the maximum blood pressure and the lower (diastolic) value is the minimum blood pressure. A high systolic blood pressure indicates hypertension. This affects one third of the adult population and is the single leading cause globally of preventable heart attacks, strokes and deaths. A significant difference between the systolic blood pressure measurements in the two arms could be indicative of a narrowing, or a stiffening, of the arteries, which can affect blood flow. These arterial changes are recognised as a further risk marker for subsequent heart attack, stroke or early death, and should be investigated for treatment.

The researchers concluded that each mmHg difference found between the two arms elevated predicted 10-year risk of one of the following occurring by one percent: new angina, a heart attack or stroke.

At the moment, both UK and European guidelines recognise a systolic difference of 15 mmHg or more between the two arms as the threshold indicative of additional cardiovascular risk. This study found that a lower threshold of 10 mmHg was clearly indicative of additional risk, which would mean that far more people should be considered for treatment if such a difference between arms is present. To this end, the research team has created a tool that is easy for clinicians to use, to establish who should be considered for treatment based on their risk, incorporating the blood pressure reading in both arms.

Research co-author Professor Victor Aboyans, head of the department of cardiology at the Dupuytren University Hospital in Limoges, France, said 'We believe that a 10-mmHg difference can now reasonably be regarded as an upper limit of normal for systolic inter-arm blood pressure, when both arms are measured in sequence during routine clinical appointments. This information should be incorporated into future guidelines and clinical practice in assessing cardiovascular risk. It would mean many more people were considered for treatment that could reduce their risk of heart attack, stroke and death.'

*Source:* Medical Brief 2020