

Associations between flavan-3-ol intake and CVD risk in the Norfolk cohort of the European Prospective Investigation into Cancer (EPIC-Norfolk)

Article

Accepted Version

Creative Commons: Attribution 3.0 (CC-BY)

Open Access

Vogiatzoglou, A., Mulligan, A. A., Bhaniani, A., Lentjes, M. A. H., McTaggart, A., Luben, R. N., Heiss, C., Kelm, M., Merx, M. W., Spencer, J. P. E. ORCID: <https://orcid.org/0000-0003-2931-7274>, Schroeter, H., Khaw, K.-T. and Kuhnle, G. G. C. ORCID: <https://orcid.org/0000-0002-8081-8931> (2015) Associations between flavan-3-ol intake and CVD risk in the Norfolk cohort of the European Prospective Investigation into Cancer (EPIC-Norfolk). *Free Radical Biology and Medicine*, 84. pp. 1-10. ISSN 0891-5849 doi: 10.1016/j.freeradbiomed.2015.03.005 Available at <https://centaur.reading.ac.uk/39640/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.1016/j.freeradbiomed.2015.03.005>

Publisher: Elsevier

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

www.reading.ac.uk/centaur

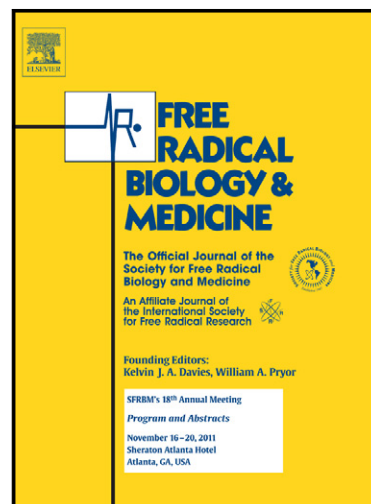
CentAUR

Central Archive at the University of Reading

Reading's research outputs online

Associations between flavan-3-ol intake and CVD risk in the Norfolk cohort of the European Prospective Investigation into Cancer (EPIC-Norfolk)

Anna Vogiatzoglou, Angela A. Mulligan, Amit Bhaniani, Marleen A.H. Lentjes, Alison McTaggart, Robert N. Luben, Christian Heiss, Malte Kelm, Marc W. Merx, Jeremy P.E. Spencer, Hagen Schroeter, Kay-Tee Khaw, Gunter G.C. Kuhnle



www.elsevier.com/locate/freeradbiomed

PII: S0891-5849(15)00117-3
DOI: <http://dx.doi.org/10.1016/j.freeradbiomed.2015.03.005>
Reference: FRB12345

To appear in: *Free Radical Biology and Medicine*

Received date: 11 October 2014
Revised date: 27 January 2015
Accepted date: 5 March 2015

Cite this article as: Anna Vogiatzoglou, Angela A. Mulligan, Amit Bhaniani, Marleen A.H. Lentjes, Alison McTaggart, Robert N. Luben, Christian Heiss, Malte Kelm, Marc W. Merx, Jeremy P.E. Spencer, Hagen Schroeter, Kay-Tee Khaw, Gunter G.C. Kuhnle, Associations between flavan-3-ol intake and CVD risk in the Norfolk cohort of the European Prospective Investigation into Cancer (EPIC-Norfolk), *Free Radical Biology and Medicine*, <http://dx.doi.org/10.1016/j.freeradbiomed.2015.03.005>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting galley proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Associations between flavan-3-ol intake and CVD risk in the Norfolk cohort of the European Prospective Investigation into Cancer (EPIC-Norfolk)

Anna Vogiatzoglou¹, Angela A. Mulligan², Amit Bhaniani², Marleen A. H. Lentjes², Alison McTaggart², Robert N. Luben², Christian Heiss³, Malte Kelm³, Marc W. Merx³, Jeremy P.E. Spencer³, Hagen Schroeter⁴, Kay-Tee Khaw^{2,5} and Gunter G. C. Kuhnle^{1,2,*}

- ¹) Department of Food & Nutritional Sciences, University of Reading, UK
- ²) Department of Public Health and Primary Care, University of Cambridge, UK
- ³) Division of Cardiology, Pulmonology and Vascular Medicine, Medical Faculty, University Düsseldorf, Germany
- ⁴) Mars, Inc., McLean, VA 22101, USA
- ⁵) University of Cambridge, School of Clinical Medicine, Clinical Gerontology Unit, Cambridge, United Kingdom
- *) Corresponding author: Department of Food & Nutritional Sciences, University of Reading, Reading RG6 6AP, UK; Tel: +44 118 378 7723; Fax: +44 118 378 7708; email: g.g.kuhnle@reading.ac.uk

Names for PubMed Indexing: Vogiatzoglou, Mulligan, Bhaniani, Lentjes, McTaggart, Luben, Heiss, Kelm, Merx, Spencer, Schroeter, Khaw, Kuhnle

Sources of funding: This project was supported by the European Union (grant 226588, “Flaviola”) and an unrestricted grant by Mars, Inc. EPIC Norfolk is supported by Cancer Research UK (SP2024-0201 and SP2024-0204) and the Medical Research Council (G9502233).

Abbreviations: epicatechin-3'-gallate (ECG), epigallocatechin (EGC), epigallocatechin-3'-gallate (EGCG), galocatechin (GC), catechin-3'-gallate (CG), European Food Safety Authority (EFSA), Degree of polymerisation (DP), 7-day-diary (7DD), food frequency

questionnaire (FFQ), cardiovascular disease (CVD), ischaemic heart disease (IHD),
European Prospective Investigation into Cancer and Nutrition (EPIC)

Abstract

Background: Dietary intervention studies suggest that flavan-3-ol intake can improve vascular function and reduce the risk of cardiovascular diseases (CVD). However, results from prospective studies failed to show a consistent beneficial effect.

Objective: To investigate associations between flavan-3-ol intake and CVD risk in the Norfolk arm of the *European Prospective Investigation into Cancer and Nutrition* (EPIC-Norfolk).

Design: Data was available from 24,885 (11,252 men; 13,633 women) participants, recruited between 1993 and 1997 into the EPIC-Norfolk study. Flavan-3-ol intake was assessed using 7-day food diaries and the *FLAVIOLA Flavanol Food Composition database*. Missing data for plasma cholesterol and vitamin C were imputed using multiple imputation. Associations between flavan-3-ol intake and blood pressure at baseline were determined using linear regression models. Associations with CVD risk were estimated using Cox regression analyses.

Results: Median intake of total flavan-3-ols was 1034 mg/d (range: 0 – 8531 mg/d) for men and 970 mg/d (0 – 6695 mg/d) for women, median intake of flavan-3-ol monomers was 233 mg/d (0 – 3248 mg/d) for men and 217 (0 – 2712 mg/d) for women. There were no consistent associations between flavan-3-ol monomer intake and baseline systolic and diastolic blood pressure (BP). After 286,147 person-years of follow up, there were 8463 cardio-vascular events and 1987 CVD related deaths; no consistent association between flavan-3-ol intake and CVD risk (HR 0.93, 95% CI: 0.87; 1.00; Q1 vs Q5) or mortality was observed (HR 0.93, 95% CI: 0.84; 1.04).

Conclusions: Flavan-3-ol intake in EPIC-Norfolk is not sufficient to achieve a statistically significant reduction in CVD risk.

Keywords: flavan-3-ols; cardio-vascular diseases; nutritional epidemiology;
EPIC Norfolk

Introduction

The medicinal use of flavan-3-ols and proanthocyanidins has a long documented history (1) and as early as 1590 in the *Florentine Codex* (2), cacao beans, a major source of flavanols, were recommended to treat heart disease (3). In recent decades, there has been an increasing interest in the physiological effects of flavan-3-ols on vascular function. While their vasculoprotective properties were at first attributed to their activity as antioxidants *in vitro*(4), recent research has shown that this is unlikely (5) and that the modulation of arterial function of flavan-3-ols is based on a nitric oxide dependent mechanism (6-8).

Dietary intervention studies with different sources of flavan-3-ols, such as cocoa-flavanols (9-11) or grape-seed extracts (12) have shown a potentially beneficial effect on vascular function. However, data from observational studies are more ambiguous. Data from anthropological research in a population with high habitual intake of cocoa, the Kuna of Panama, suggest that flavan-3-ol intake can improve vascular function (13), but results from the *Nurses Health Studies* and *Health Professionals Follow-up study* (14-16), the *Iowa Women's Health Study* (17), the *Cancer Prevention Study* (18) and the *Zutphen Elderly Study* (19) have not shown any consistent associations between flavan-3-ol intake and CVD risk or risk factors such as blood pressure. Studies investigating associations between CVD risk and the intake of flavan-3-ol rich foods, in particular chocolate, did show beneficial effects (20-22), but the flavan-3-ol contribution from chocolate in the European population is very small (23) and it is likely that self-reported chocolate consumption acts mainly as a surrogate marker for a yet unidentified factor.

The results from intervention studies suggest that flavan-3-ols can reduce the risk for CVD. This is of great importance in the context of public health, as CVD are

responsible for approximately 30% of all deaths globally (24). However, the ambiguity of results from observational studies raises several questions, in particular how the apparent contradiction with intervention studies can be explained and whether these data can be easily translated to the general public.

The primary objective of this study was to investigate associations between flavan-3-ol intake and CVD risk in the Norfolk arm of the *European Prospective Investigation into Cancer and Nutrition* (EPIC-Norfolk), and to compare these results with data from previous studies as well as dietary intervention studies in the context of primary prevention of CVD. The EPIC-Norfolk cohort is particularly well placed to investigate these associations, as it has the widest range and highest intake of flavan-3-ol intake among all EPIC cohorts (25) and a wider range of intake than cohorts investigated previously. Furthermore, we have assessed intake using 7-day-diaries (7DD) and a newly developed food composition database (23), which provide a more accurate estimate of dietary intake.

Subjects and Methods

Study population

Between 1993 and 1997, approximately 30,000 men and women between 40 and 75 years were recruited for the *European Prospective Investigation into Cancer and Nutrition* (EPIC) Norfolk study from a general practitioners' database (26). 25,639 participants attended a health examination. Diet was assessed by 7DD. The first day of the diary was completed as a 24-hour recall (24HDR) with a trained interviewer and the remainder completed during subsequent days. Diary data were entered using the in-house dietary assessment software DINER – Data into Nutrients for Epidemiological Research (27); data were checked and calculated using DINERMO (28). Health and lifestyle characteristics, including data on smoking, alcohol consumption, social class, family medical history, exercise and reproductive history, were assessed by questionnaire. Height and weight measurements were collected following a standardised protocol as part of a health check conducted by trained research nurses. Physical activity, representing occupational and leisure activity, was assessed using a validated questionnaire (29). Blood pressure was measured by using a non-invasive oscillometric blood pressure monitor (Acutorr; Datascope Medical, Huntingdon, United Kingdom) after the participant had been seated in a comfortable environment for 5 minutes. The arm was horizontal and supported at the level of the mid-sternum; the mean of two readings was used for analysis. Acutorr readings were checked against those from sphygmomanometers every 6 months. The study was approved by the Norwich District Health Authority Ethics Committee, and all participants gave signed informed consent.

Non-fasting blood samples were taken by vein puncture at baseline health check and stored in serum tubes. Serum levels of total cholesterol were measured on fresh

samples with the RA 1000 auto-analyzer (Bayer Diagnostics, Basingstoke, United Kingdom). Plasma Vitamin C was measured using a fluorometric assay as described previously (30). Baseline data for 25,639 participants (11,607 men; 14,032 women) was available. Participants with data processing errors (4 men), incidence date before the start of the study (10 men; 4 women) and missing dietary data (73 men; 62 women) were excluded. Furthermore, participants with self-reported energy intake in the bottom (p1) or top (p99) sex-specific percentile (men: 4.6 MJ – 15.3 MJ; women: 3.5 MJ – 11.4 MJ) (230 men; 279 women), missing BMI (20 men; 23 women) and missing endpoint data (systolic blood pressure; 24 men; 36 women) were excluded, resulting in a sample size of 24,885 (11,252 men; 13,633 women; see also Figure 1).

Incident CVD event and mortality

The primary endpoint of this study was any first CVD event (defined as ICD 410–448 (ICD 9) or ICD I10–I79 (ICD 10), which includes coronary heart disease (410–414 (ICD 9) or I20–I25 (ICD 10)), stroke (430–438 (ICD 9) or I60–I69 (ICD 10)) and cardiac failure (428 (ICD 9) or I50 (ICD 10)) and other vascular causes) during follow-up. All participants were followed up for fatal and non-fatal CVD events, and the present study includes events until 31 March 2009, covering 11.1 ± 3.7 years (mean \pm SD) of follow up. Cause-specific hospital admission was determined via ENCORE (East Norfolk Commission Record, the hospital admissions database kept by the East Norfolk Health Commission) (31) with the individuals' unique National Health Service number. All individuals were flagged by the UK Office of National Statistics (ONS) for death certification and trained nosologists coded death certificates according to the International Classification of Disease (ICD). Death certificates and ENCORE show high accuracy in correctly identifying incident disease, as previously shown in EPIC-Norfolk for incident stroke (31).

Flavan-3-ol intake

We estimated flavan-3-ol intake by using the *FLAVIOLA Flavanol Food Composition database*, which was developed for the FLAVIOLA project(23). The database contains food composition data for approximately 3,000 food items and is based on the US Department of Agriculture database for the Flavonoid Content of Selected Foods (32) and the proanthocyanidins (PA) content of Selected Foods (33), expanded with values from Phenol-Explorer, a comprehensive database on the polyphenol content of foods (34). These databases are the most up-to-date databases on flavonoids and polyphenols, and include information for 500, 205, and 456 food items for flavonoids (32), PA (33) and polyphenols (34), respectively. On the basis of these data, we determined the flavan-3-ol content of foods by substitution and calculations to reflect edible portions, food preparation methods and recipe break down (see (35) for the methodology). Flavan-3-ol intake was determined for the following groups of flavan-3-ols (**Table 1**): flavan-3-ols monomers [(-)-epicatechin, (-)-epicatechin-3-gallate, (+)-catechin, (-)-epigallocatechin, (-)-epigallocatechin-3-gallate, (+)-gallocatechin, (+)-catechin-3-gallate], theaflavins (theaflavin, thearubigins, theaflavin-3,3'-digallate, theaflavin-3'-gallate, theaflavin-3-gallate), and PA (dimers, trimers, 4-6mers, 7-10mers, >10mers).

Data analysis

Missing data

Supplemental Table 1 shows a summary of missing data. For categorical data, an additional value of “missing” was created. For continuous variables (cholesterol and plasma vitamin C), missing values were assumed to be missing at random and were imputed using multiple imputation (n=5) with univariate regression models.

Descriptive statistics

Descriptive characteristics of the study population were summarised using mean (plus or minus standard deviation) for normally distributed continuous variables, frequency and percentages for categorical variables and geometric means (standard deviation) for non-normally distributed continuous variables.

Association between flavan-3-ol intake and blood pressure

Associations between flavan-3-ol intake and systolic blood pressure were investigated using linear regression models; dietary intake data were divided into sex-specific quintiles. Log-transformed values were used for the dependent variable (systolic blood pressure), as well as for BMI (body weight/height² in kg/m²) and the intake of energy, sodium, potassium, fat and saturated fat. In initial analyses (model 1), intake was adjusted by age, BMI (kg/m²) and energy intake (kJ/d). A parsimonious model was developed using a stepwise selection approach (only co-variables with a p-value < 0.2 were included in the model) using the following co-variables: BMI (kg/m²), energy intake (kJ/d), plasma vitamin C (μmol/L), fibre, total fat and saturated fat consumptions (g/day), alcohol consumption (0 g/d, <15 g/d, ≥15 g/d) and the following categorical variables - physical activity (inactive, moderately inactive, moderately active, active), smoking status (former, current, never), social class (professional, managerial, skilled (manual/non-manual), semi-skilled, unskilled), marital status (single, married, widowed, divorced, separated), education (none, O-level, A-level, degree), self-reported history of stroke, myocardial infarction and diabetes at baseline, family history of myocardial infarction, use of anti-hypertensive and lipid lowering drugs; additionally in women hormone replacement therapy (current, former, never) and menopausal status (pre, peri and post). The parsimonious model included age, BMI, energy intake, plasma vitamin C, dietary intake of fibre, fat, saturated fat and alcohol and the following categorical variables:

physical activity, smoking status, marital status, education, use of anti-hypertensive drugs and self-reported history of stroke, myocardial infarction and diabetes at baseline, family history of myocardial infarction and – for women – menopausal status and hormone replacement therapy. Tests for linear trend were conducted by including the median value of each quintile in the model.

Association between flavan-3-ol intake and CVD risk

Associations between quintiles of flavan-3-ol intake and CVD risk and mortality were analysed using a Cox regression analysis to investigate hazard ratios and 95% confidence intervals. Log-transformed values were used for systolic blood pressure, BMI and the intakes of energy, sodium, potassium, fat and saturated fat.

Associations were first analysed adjusting for BMI and energy intake only. A parsimonious model was then developed as described above, and it included the following variables: BMI, energy intake, systolic blood pressure, plasma cholesterol, plasma vitamin C, intake of fibre, potassium, sodium, fat, saturated fat and alcohol, and the following categorical variables - physical activity, smoking status, education, self-reported history of stroke, myocardial infarction and diabetes at baseline, family history of myocardial infarction, use of anti-hypertensive and lipid lowering drugs; additionally in women use of hormone replacement therapy. Tests for linear trend were conducted by including the median value of each quintile in the model.

Sensitivity analyses

In post-hoc sensitivity analyses, we investigated associations in participants healthy at baseline (no self-reported use of anti-hypertensive drugs, no self-reported incidence of diabetes mellitus, myocardial infarction or stroke), those at high risk of CVD (10-year CVD risk 20% higher than normal based on Framingham risk score(36)) and those with at least three days of dietary data.

Strobe checklist

The STROBE checklist for cohort studies(37) has been completed for this study.

Statistical analyses

All analyses were conducted with Stata (version 11 for Mac OS X; Stata Corp, College Station, Texas, USA).

Results

Study population and flavan-3-ol monomer intake

Data were available for 11,252 men and 13,633 women, of which 8176 were post-menopausal. Baseline characteristics of the cohort and dietary information by sex-specific quintile of flavan-3-ol monomer intake are shown in **Supplemental Table 2**.

Mean age of the participants at baseline was 59 years (range: 39 – 79 years) for men and 58 years (39 – 78 years) for women. Median intake of total flavan-3-ols was 1034 mg/d (0 – 8531 mg/d) for men and 970 mg/d (0 – 6695 mg/d) for women, median intake of flavan-3-ol monomers was 233 mg/d (0 – 3248 mg/d) for men and 217 (0 – 2712 mg/d) for women (see **Table 2** for more details). While there were significant differences in many baseline characteristics between sex-specific quintiles of flavan-3-ol monomer intake, these differences did not follow a consistent trend.

The relative composition of dietary flavan-3-ol intake was consistent in the top four quintiles of flavan-3-ol intake (Q2 to Q5), with gallated compounds being the main contributor to total intake of monomers (**Figure 2**; the same pattern was observed for women and post-menopausal women) and theaflavins the main type of oligomeric flavan-3-ols. However, in participants with low flavan-3-ol intake (Q1), (–)-epicatechin and (+)-catechin were the main sources of flavan-3-ol monomers and proanthocyanidins the main type of oligomeric flavan-3-ols consumed. It was also noticeable that the relative composition of dietary flavan-3-ols was more variable in the bottom quintile.

Associations between flavan-3-ol intake, CVD risk and mortality

In women, flavan-3-ol intake was not associated with CVD risk or mortality and these results were consistent when using different models and also in sensitivity analyses

(**Table 3**). In men, flavan-3-ol intake was not associated with mortality and the risk of stroke. However, the association between flavan-3-ol intake and both, IHD risk and total CVD risk, was more complex and followed a J-shaped curve: low and medium intake (Q2 and Q3) were both associated with a reduced risk. The risk estimates for quintiles two to five of flavan-3-ol intake were not significantly different and there was no linear trend.

Post-hoc sensitivity analyses with healthy individuals, individuals with a raised 10-year CVD risk and those with at least three days of diary data, showed no materially different associations. In sensitivity analyses, we investigated the association between specific groups of flavan-3-ols and CVD risk: no material differences were observed between different types of flavan-3-ols (**Figure 3**).

Associations between flavan-3-ol intake and blood pressure

Supplemental Table 4 shows the results of regression analyses between flavan-3-ol intake and systolic and diastolic blood pressure (log-transformed) at baseline. There were no consistent significant association between flavan-3-ol intake and baseline blood pressure.

Discussion

The EPIC-Norfolk cohort is well positioned for a detailed investigation of the association between flavan-3-ol intake and CVD risk, in particular because of the prospective design of the study, the wide range of flavan-3-ol intake and the detailed dietary information available from 7-day diet diaries. In combination with the newly developed *FLAVIOLA Flavanol Food Composition Database*, this provides the most accurate estimate of flavan-3-ol consumption in a prospective cohort currently available. There are some limitations, in particular the known limitations of self-reported dietary assessment and the accuracy of phytochemical intake based on food composition tables. We have also conducted multiple comparisons without adjustment, which should be considered when interpreting results.

The estimated flavan-3-ol monomer intake in this study was higher than in comparable studies conducted previously (**Supplemental Table 4**). Despite the wider range of intake, we have not found any consistent association between flavan-3-ol monomer intake and systolic blood pressure at baseline or risk of CVD, and our data are corroborated by results from previous studies (15, 17, 18, 20, 38) (**Figure 4** and **Supplemental Table 4**).

It was noticeable that the association between flavan-3-ol intake and CVD risk followed a J-shaped curve with the lowest relative risk in quintile 2, and this pattern has also been observed in previous studies (15, 17, 18). Our data show differences in the relative composition of flavan-3-ols consumed between those in the bottom quintile of intake and other participants (Figure 2). While data on flavan-3-ol composition in the bottom quintile should be interpreted carefully, because of the error associated with the assessment of such small amounts, it is likely that this

difference in relative flavan-3-ol composition is an explanation for the J-shaped associations we observed.

The absence of consistent statistically significant associations between flavan-3-ol intake and CVD risk is noteworthy in light of data from dietary intervention studies, which suggest vasculoprotective effects of dietary flavan-3-ols. Indeed, several meta-analyses have shown a significant reduction in systolic blood pressure (3 mm Hg to 5 mm Hg) for interventions with cocoa flavanols (10, 11) and smaller reductions with other flavan-3-ol rich foods such as tea (39) or grape seed extract (40). A reduction of systolic blood pressure by 3 to 5 mm Hg is comparable to the effect that could be achieved by a modest reduction in salt intake (41). Projections based on the DASH diet and data from NHANES estimate that such a reduction in blood pressure could translate into approximately 3% – 4% fewer CHD events over 10 years (42).

However, a direct comparison of results from intervention studies with data from this study is limited because observational epidemiological studies investigate by their very nature chronic effects, whereas most intervention studies focused on acute and sub-acute effects (see **Supplemental Table 5** for a summary of dietary intervention studies), and there is a paucity of data on chronic effects. There is also considerable ambiguity regarding the type of flavan-3-ols used. While in observational studies all but one, the Iowa Women's Health Study (17), used flavan-3-ol monomers as defined here (Table 1), this is different for dietary intervention studies. In some intervention studies, flavan-3-ols are not characterised by compound-specific analyses, but only by their source. However, differences in growing conditions, harvesting, processing and storage can result in a large variation of flavan-3-ol content in foods (43-46). For these reasons, the actual flavan-3-ol content, and their relative composition, are often not known. This is particularly important because so far only the intake of one member of the cocoa flavanol group, (–)-epicatechin, has directly and causally been

linked to the modulation of vascular function (7, 8). The absorption of oligomeric proanthocyanidins, important constituents of cocoa flavanols and grape seed extracts, is negligible and they do not contribute to the pool of flavan-3-ol metabolites (47).

Using food composition tables, we have estimated the (–)-epicatechin intake in dietary intervention studies, assuming that cocoa flavanols consist of approximately 10% (–)-epicatechin, 7% (+)-catechin and 83% proanthocyanidins (DP 2 – 10). A comparison of intakes used in intervention studies and the intake of the population in EPIC-Norfolk (**Figure 5**) shows clearly that intakes in most studies were considerably higher, and it is likely that (–)-epicatechin consumption in EPIC-Norfolk, and other cohorts, is too low to have an effect on overall CVD risk. Indeed, a recent meta-analysis has suggested that an effect on systolic blood pressure can only be observed with intakes in excess of 50 mg/d (9) of (–)-epicatechin, but more than 90% of the EPIC Norfolk population do not consume this much, even though the Norfolk cohort of EPIC has the highest (–)-epicatechin intake of all EPIC cohorts (25) and participants consume considerably more than the general European public (mean intake in the EU: 13.5 mg/d) (23). A comparison of mean (–)-epicatechin in different European countries with age-adjusted CHD mortality does not suggest a significant association (Figure 6). These data suggest that (–)-epicatechin intake in the general public is too low to achieve an overall reduction of cardio-vascular disease risk.

In 2012, EFSA approved a health claim for cocoa flavanols, based on an unspecified degree of increase in flow-mediated dilation (FMD) (48) after cocoa flavanol intake. The scientific opinion underlying this claim states that 200 mg/d of cocoa flavanols can achieve improved vascular function. Independent of these findings, studies have shown that a FMD increase of 1% to 1.5% could translate in a reduction of CVD events by 10% (49), and such a reduction has been achieved in

dietary intervention studies with cocoa flavanols (9). In EPIC Norfolk, approximately 25% of participants consumed 200 mg/d or more of cocoa flavanols. However, there was no significant difference in CVD risk between those who met the 200 mg/d recommendation and those who did not (HR 0.97; 95% CI: 0.89; 1.04 for men; HR 0.96; 95% CI: 0.89; 1.05 for women). When basing the intake recommendation on the known bioactive constituent of cocoa-flavanols, (–)-epicatechin, more than 70% of participants met or exceed the recommendations of 20 mg/d. We observed no statistically significant association between intake above 20 mg/d and CVD risk in women (HR 1.00; 95% CI: 0.94; 1.08). However, this analysis is more complex in men as all participants with an (–)-epicatechin intake of less than 20 mg/d are in the bottom quintile of (–)-epicatechin and flavan-3-ol monomer intake. The small, but significant, association between CVD risk and (–)-epicatechin intake found for men (HR 0.92; 95% CI: 0.86; 0.99) could therefore also be explained by differences in dietary patterns as discussed above and needs to be confirmed in further studies.

Many dietary recommendations focus primarily on the prevention of deficiency diseases (1), but there is an increasing interest in also focusing on *bioactives* in the context of disease risk reduction and primary prevention (50). There is accumulating evidence for the vasculoprotective effect of dietary flavan-3-ols and the observed effect on vascular function suggests that an adequate intake could result in a 10% reduction of CVD risk in the general public. This has important implications in the context of primary prevention of CVD and evidence-based dietary recommendations. So far, it is not yet clear what an adequate intake is: neither is it clear whether it should be based on individual compounds, such as (–)-epicatechin, or a group of compounds such as cocoa flavanols, nor is it clear which amount should be recommended. Nevertheless, the data presented here clearly shows that the habitual intake in Europe or the US is unlikely to have any significant effect on CVD risk on a

population basis. Indeed, for such a reduction to occur, an intake considerably higher than the amount currently consumed by the general public is necessary.

Based on these data and previous investigations, it is essential that new methods are developed that enable further research to study more comprehensively the influences of age, sex and dietary background. Specifically in the context of observational population-based studies, improved dietary assessment methods are crucial for a better understanding of associations between intake and CVD risk. Nutritional biomarkers can provide such methods, but there is currently no commonly accepted, validated biomarker of flavan-3-ol intake, which meets the recommendations of the 2010 IOM biomarker report (51). The short half-life plasma in of structurally-related flavan-3-ol metabolites (52) restricts their use to the assessment of acute intake. We have hypothesised that flavan-3-ol metabolites of the colonic microbiome, such as 5-(3,4-dihydroxyphenyl)- γ -valerolactone (γ -VL) (53), which have a considerably longer biological half-life and are present in higher concentrations (47), are more suitable as nutritional biomarkers of flavan-3-ol intake. In order to investigate these compounds on a large scale, we have developed a validated analytical method which can be automated for high-throughput analysis. We are currently applying it to a cohort of approximately 25,000 participants to assess colonic metabolites as nutritional biomarkers against the IOM criteria. Such a biomarker will then allow us a much more accurate estimate of intake.

Only a combination of data from observational and intervention studies will ultimately enable a comprehensive assessment of the impact of flavan-3-ol intake in the context of primary prevention, public health and evidence-based dietary recommendations.

Acknowledgements

We thank all EPIC-Norfolk study participants and staff for their contribution to the study. We thank the members of the FLAVIOLA consortium for their critical review of the manuscript. The present study was supported by the EU (grant 226588, 'Flaviola') and an unrestricted grant from Mars, Inc. Mars, Inc. had no role in the design and analysis of the study or in the writing of this article. EPIC Norfolk is supported by Cancer Research UK (SP2024-0201 and SP2024-0204) and the Medical Research Council (G9502233).

The responsibilities of the authors were as follows: GGCK conducted the statistical analyses, wrote the manuscript and had primary responsibility for the final content; AV, AAM, MAHL, AMcT were responsible for calculating flavan-3-ol intake and data-checking; AB, RNL were responsible for follow-up and identification of cases and data processing; CH, MK, MWM, JPES, HS, K-TK (principle investigator of EPIC Norfolk) contributed to the manuscript. All authors read and approved the final manuscript.

H. S. is employed by MARS, Inc., a member of the FLAVIOLA research consortium and a company engaged in flavanol research and flavanol-related commercial activities. None of the other authors has a conflict of interest to declare.

References

- Schroeter H, Heiss C, Spencer JPE, Keen CL, Lupton JR, Schmitz HH. Recommending flavanols and procyanidins for cardiovascular health: Current knowledge and future needs. *Molecular Aspects of Medicine* 2010;31(6):546-57. doi: <http://dx.doi.org/10.1016/j.mam.2010.09.008>.
- de Sahagún B. Historia general de las cosas de Nueva España, 1590.
- Dillinger TL, Barriga P, Escarcega S, Jimenez M, Salazar Lowe D, Grivetti LE. Food of the gods: cure for humanity? A cultural history of the medicinal and ritual use of chocolate. *J Nutr* 2000;130(8S Suppl):2057S-72S.
- Keen CL, Holt RR, Oteiza PI, Fraga CG, Schmitz HH. Cocoa antioxidants and cardiovascular health. *Am J Clin Nutr* 2005;81(1 Suppl):298S-303S.
- Heiss C, Keen CL, Kelm M. Flavanols and cardiovascular disease prevention. *European heart journal* 2010;31(21):2583-92. doi: 10.1093/eurheartj/ehq332.
- Heiss C, Kleinbongard P, Dejam A, Perré S, Schroeter H, Sies H, Kelm M. Acute Consumption of Flavanol-Rich Cocoa and the Reversal of Endothelial Dysfunction in Smokers. *Journal of the American College of Cardiology* 2005;46(7):1276-83. doi: 10.1016/j.jacc.2005.06.055.
- Schroeter H, Heiss C, Balzer J, Kleinbongard P, Keen C, Hollenberg N, Sies H, Kwik-Urbe C, Schmitz H, Kelm M. (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. *Proceedings of the National Academy of Sciences of the United States of America* 2006;103(4):1024-9.
- Loke WM, Hodgson JM, Proudfoot JM, McKinley AJ, Puddey IB, Croft KD. Pure dietary flavonoids quercetin and (-)-epicatechin augment nitric oxide products and reduce endothelin-1 acutely in healthy men. *Am J Clin Nutr* 2008;88(4):1018-25.
- Hooper L, Kay C, Abdelhamid A, Kroon PA, Cohn JS, Rimm EB, Cassidy A. Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: a systematic review and meta-analysis of randomized trials. *American Journal of Clinical Nutrition* 2012;95(3):740-51. doi: 10.3945/ajcn.111.023457.
- Ried K, Sullivan T, Fakler P, Frank OR, Stocks NP. Effect of cocoa on blood pressure. *Cochrane Database of Systematic Reviews* 2012(8):1-83. doi: 10.1002/14651858.CD008893.pub2.
- Ellinger S, Reusch A, Stehle P, Helfrich H-P. Epicatechin ingested via cocoa products reduces blood pressure in humans: a nonlinear regression model with a Bayesian approach. *American Journal of Clinical Nutrition* 2012;95(6):1365-77. doi: 10.3945/ajcn.111.029330.
- Feringa HHH, Laskey DA, Dickson JE, Coleman CI. The Effect of Grape Seed Extract on Cardiovascular Risk Markers: A Meta-Analysis of Randomized Controlled Trials. *Journal of the American Dietetic Association* 2011;111(8):1173-81. doi: <http://dx.doi.org/10.1016/j.jada.2011.05.015>.
- Hollenberg NK, Martinez G, McCullough M, Meinking T, Passan D, Preston M, Rivera A, Taplin D, Vicaria-Clement M. Aging, acculturation, salt intake, and hypertension in the Kuna of Panama. *Hypertension* 1997;29(1 Pt 2):171-6. doi: 10.1161/01.HYP.29.1.171.
- Cassidy A, Rimm EB, O'Reilly EJ, Logroscino G, Kay C, Chiuve SE, Rexrode KM. Dietary flavonoids and risk of stroke in women. *Stroke; a journal of cerebral circulation* 2012;43(4):946-51. doi: 10.1161/STROKEAHA.111.637835.
- Cassidy A, O'Reilly EJ, Kay C, Sampson L, Franz M, Forman J, Curhan G, Rimm EB. Habitual intake of flavonoid subclasses and incident hypertension in adults. *American Journal of Clinical Nutrition* 2011;93(2):338-47. doi: 10.3945/ajcn.110.006783.
- Samieri C, Sun Q, Townsend MK, Rimm EB, Grodstein F. Dietary flavonoid intake at midlife and healthy aging in women. *The American Journal of Clinical Nutrition* 2014;100(6):1489-97. doi: 10.3945/ajcn.114.085605.
- Mink PJ, Scrafford CG, Barraj LM, Harnack L, Hong C-P, Nettleton JA, Jacobs DR. Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. *The American journal of clinical nutrition* 2007;85(3):895-909.
- McCullough ML, Peterson JJ, Patel R, Jacques PF, Shah R, Dwyer JT. Flavonoid intake and cardiovascular disease mortality in a prospective cohort of US adults. *American Journal of Clinical Nutrition* 2012;95(2):454-64. doi: 10.3945/ajcn.111.016634.

19. Arts IC, Hollman PC, Feskens EJ, Bueno de Mesquita HB, Kromhout D. Catechin intake might explain the inverse relation between tea consumption and ischemic heart disease: the Zutphen Elderly Study. *Am J Clin Nutr* 2001;74(2):227-32.
20. Buijsse B, Feskens EJM, Kok FJ, Kromhout D. Cocoa intake, blood pressure, and cardiovascular mortality: the Zutphen Elderly Study. *Archives of internal medicine* 2006;166(4):411-7. doi: 10.1001/.411.
21. Buijsse B, Weikert C, Drogan D, Bergmann M, Boeing H. Chocolate consumption in relation to blood pressure and risk of cardiovascular disease in German adults. *European heart journal* 2010;31(13):1616-23. doi: 10.1093/eurheartj/ehq068.
22. Larsson SC, Virtamo J, Wolk A. Chocolate consumption and risk of stroke: a prospective cohort of men and meta-analysis. *Neurology* 2012;79(12):1223-9. doi: 10.1212/WNL.0b013e31826aacfa.
23. Vogiatzoglou A, Mulligan AA, Luben RN, Lentjes MAH, Heiss C, Kelm M, Merx M, Spencer JPE, Schroeter H, Kuhnle GGC. Assessment of the dietary intake of total flavan-3-ols, monomeric flavan-3-ols, proanthocyanidins and theaflavins in the European Union. *British Journal of Nutrition* 2014;111(8):1463-73.
24. Global status report on noncommunicable diseases 2010. Geneva, Switzerland: World Health Organization, 2011.
25. Knaze V, Zamora-Ros R, Lujan-Barroso L, Romieu I, Scalbert A, Slimani N, Riboli E, van Rossum CT, Bueno-de-Mesquita HB, Trichopoulou A, et al. Intake estimation of total and individual flavan-3-ols, proanthocyanidins and theaflavins, their food sources and determinants in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Br J Nutr* 2012;108(6):1095-108. doi: 10.1017/S0007114511006386.
26. Day NE, Oakes S, Luben RN, Khaw K-T, Bingham SA, Welch AA, Wareham NJ. EPIC-Norfolk: study design and characteristics of the cohort. *European Prospective Investigation of Cancer. British Journal of Cancer* 1999;80 Suppl 1:95-103.
27. Welch AA, McTaggart A, Mulligan AA, Luben RN, Walker N, Khaw K-T, Day NE, Bingham SA. DINER (Data Into Nutrients for Epidemiological Research) - a new data-entry program for nutritional analysis in the EPIC-Norfolk cohort and the 7-day diary method. *Public Health Nutrition* 2001;4(6):1253-65.
28. Lentjes MAH, McTaggart A, Mulligan AA, Powell NA, Parry-Smith D, Luben RN, Bhaniani A, Welch AA, Khaw K-T. Dietary intake using 7-day diaries in British men and women in the EPIC-Norfolk Study: a focus on methodological issues. *British Journal of Nutrition* 2013;in press.
29. Wareham NJ, Jakes R, Rennie K, Mitchell J, Hennings S, Day NE. Validity and repeatability of the EPIC-Norfolk Physical Activity Questionnaire. *International Journal of Epidemiology* 2002;31(1):168-74.
30. Khaw K-T, Bingham S, Welch A, Luben R, Wareham N, Oakes S, Day N. Relation between plasma ascorbic acid and mortality in men and women in EPIC-Norfolk prospective study: a prospective population study. *The Lancet* 2001;357(9257):657-63.
31. Sinha S, Myint PK, Luben RN, Khaw KT. Accuracy of death certification and hospital record linkage for identification of incident stroke. *BMC medical research methodology* 2008;8:74. doi: 10.1186/1471-2288-8-74.
32. USDA. USDA Database for the Flavonoid Content of Selected Foods. Beltsville, MD: US Department of Agriculture, 2011.
33. USDA. USDA Database for the Proanthocyanidin Content of Selected Foods. Beltsville, MD: US Department of Agriculture, 2007.
34. Neveu V, Perez-Jimenez J, Vos F, Crespy V, du Chaffaut L, Mennen L, Knox C, Eisner R, Cruz J, Wishart D, et al. Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *Database (Oxford)* 2010;2010:bap024. doi: 10.1093/database/bap024.
35. Mulligan AA, Kuhnle GGC, Lentjes MA, van Scheltinga V, Powell NA, McTaggart A, Bhaniani A, Khaw K-T. Intakes and sources of isoflavones, lignans, enterolignans, coumestrol and soya-containing foods in the Norfolk arm of the European Prospective Investigation into Cancer and Nutrition (EPIC-Norfolk), from 7 d food diaries, using a newly updated database. *Public Health Nutr* 2012;16(8):1454-62. doi: 10.1017/S1368980012003904.
36. D'Agostino RB, Sr., Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, Kannel WB. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation* 2008;117(6):743-53. doi: 10.1161/CIRCULATIONAHA.107.699579.
37. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, Initiative S. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)

- statement: guidelines for reporting observational studies. *Lancet* 2007;370(9596):1453-7. doi: 10.1016/S0140-6736(07)61602-X.
38. Arts IC, Jacobs DR, Harnack LJ, Gross M, Folsom AR. Dietary catechins in relation to coronary heart disease death among postmenopausal women. *Epidemiology* (Cambridge, Mass) 2001;12(6):668-75.
 39. Hartley L, Flowers N, Holmes J, Clarke A, Stranges S, Hooper L, Rees K. Green and black tea for the primary prevention of cardiovascular disease. *The Cochrane database of systematic reviews* 2013;6:CD009934. doi: 10.1002/14651858.CD009934.pub2.
 40. Feringa HH, Laskey DA, Dickson JE, Coleman CI. The effect of grape seed extract on cardiovascular risk markers: a meta-analysis of randomized controlled trials. *J Am Diet Assoc* 2011;111(8):1173-81. doi: 10.1016/j.jada.2011.05.015.
 41. He FJ, MacGregor GA. Effect of modest salt reduction on blood pressure: a meta-analysis of randomized trials. Implications for public health. *J Hum Hypertens* 2002;16(11):761-70. doi: 10.1038/sj.jhh.1001459.
 42. Erlinger TP, Vollmer WM, Svetkey LP, Appel LJ. The potential impact of nonpharmacologic population-wide blood pressure reduction on coronary heart disease events: pronounced benefits in African-Americans and hypertensives. *Prev Med* 2003;37(4):327-33.
 43. Kuhnle GGC, Dell'Aquila C, Runswick SA, Bingham SA. Variability of phytoestrogen content in foods from different sources. *Food Chemistry* 2009;113:1184-7.
 44. Ottaviani JI, Momma TY, Heiss C, Kwik-Urbe C, Schroeter H, Keen CL. The stereochemical configuration of flavanols influences the level and metabolism of flavanols in humans and their biological activity in vivo. *Free radical biology & medicine* 2011;50(2):237-44. doi: 10.1016/j.freeradbiomed.2010.11.005.
 45. Andres-Lacueva C, Monagas M, Khan N, Izquierdo-Pulido M, Urpi-Sarda M, Permanyer J, Lamuela-Raventos RM. Flavanol and flavonol contents of cocoa powder products: influence of the manufacturing process. *J Agric Food Chem* 2008;56(9):3111-7. doi: 10.1021/jf0728754.
 46. Hurst WJ, Krake SH, Bergmeier SC, Payne MJ, Miller KB, Stuart DA. Impact of fermentation, drying, roasting and Dutch processing on flavan-3-ol stereochemistry in cacao beans and cocoa ingredients. *Chemistry Central journal* 2011;5:53. doi: 10.1186/1752-153X-5-53.
 47. Ottaviani JI, Kwik-Urbe C, Keen CL, Schroeter H. Intake of dietary procyanidins does not contribute to the pool of circulating flavanols in humans. *Am J Clin Nutr* 2012;95(4):851-8. doi: 10.3945/ajcn.111.028340.
 48. EFSA Panel on Dietetic Products NaAN. Scientific Opinion on the substantiation of a health claim related to cocoa flavanols and maintenance of normal endothelium-dependent vasodilation pursuant to Article 13(5) of Regulation (EC) No 1924/2006. *EFSA Journal* 2012;10(7):2809-30.
 49. Inaba Y, Chen JA, Bergmann SR. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. *The international journal of cardiovascular imaging* 2010;26(6):631-40. doi: 10.1007/s10554-010-9616-1.
 50. Lupton JR, Atkinson SA, Chang N, Fraga CG, Levy J, Messina M, Richardson DP, van Ommen B, Yang Y, Griffiths JC, et al. Exploring the benefits and challenges of establishing a DRI-like process for bioactives. *Eur J Nutr* 2014;53 Suppl 1:1-9. doi: 10.1007/s00394-014-0666-3.
 51. Institute of Medicine (US) Committee on Qualification of Biomarkers and Surrogate Endpoints in Chronic Disease. Evaluation of Biomarkers and Surrogate Endpoints in Chronic Disease. In: Micheel C, Ball J, eds. Washington, DC, 2010.
 52. Ottaviani JI, Momma TY, Kuhnle GK, Keen CL, Schroeter H. Structurally related (-)-epicatechin metabolites in humans: Assessment using de novo chemically synthesized authentic standards. *Free Radical Biology and Medicine* 2012(in press). doi: 10.1016/j.freeradbiomed.2011.12.010.
 53. Tzounis X, Vulevic J, Kuhnle GGC, George T, Leonczak J, Gibson GR, Kwik-Urbe C, Spencer JPE. Flavanol monomer-induced changes to the human faecal microflora. *The British journal of nutrition* 2008;99(4):782-92. doi: 10.1017/S0007114507853384.
 54. Nichols M, Townsend N, Luengo-Fernandez R, Leal J, Gray A, Scarborough P, Rayner M. *European Cardiovascular Disease Statistics 2012*. Brussels: European Heart Network, 2012.

Table 1: Definition of different types of flavan-3-ols used in this study.

	Flavan-3-ols	Flavan-3-ol Monomer	Cocoa flavanols
(-)-Epicatechin	X	X	X
(+)-Catechin	X	X	X
Gallated forms [†]	X	X	X
Proanthocyanidins	X		X [‡]
Thearubigins and Theaflavins [§]	X		

[†]epicatechin-3'-gallate, epigallocatechin, epigallocatechin-3'-gallate, galocatechin, catechin-3'-gallate; [‡] includes only proanthocyanidins up to a degree of polymerisation of 10; [§]theaflavin, thearubigins, theaflavin-3,3'-digallate, theaflavin-3'-gallate, theaflavin-3-gallate

Table 2: Baseline characteristics (mean and SD) and baseline intake (median and range) of 11,252 men and 13,633 women in EPIC Norfolk.

	Men	Women	Post-menopausal women
n	11252	13633	8176
Age [years]	59 (9)	58 (9)	64 (7)
BMI [kg/m ²]	27 (3)	26 (4)	27 (4)
Cholesterol [mmol/L]	6.0 (1.1)	6.3 (1.2)	6.6 (1.2)
Systolic BP [mmHg]	137.5 (17.7)	134.0 (18.9)	137.5 (17.7)
Diastolic BP [mmHg]	84.4 (11.1)	81.0 (11.1)	82.8 (11.2)
Flavan-3-ol intake in mg/d			
Flavan-3-ols	1034 (0 – 8531)	970 (0 – 6695)	981 (0 – 6695)
Flavan-3-ol monomers	233 (0 – 3248)	217 (0 – 2712)	219 (0 – 2712)
Epicatechin	27 (0 – 225)	25 (0 – 198)	25 (0 – 198)
Catechin	20 (0 – 108)	18 (0 – 84)	17 (0 – 84)
Gallated compounds	183 (0 – 2936)	172 (0 – 2453)	176 (0 – 2453)
Proanthocyanidins [†]	138 (0 – 2082)	136 (0 – 1403)	134 (0 – 1359)
Theaflavins	644 (0 – 6095)	601 (0 – 4876)	612 (0 – 4876)
Cocoa flavanols [†]	154 (0 – 1561)	144 (0 – 1118)	142 (0 – 1060)

[†]With a degree of polymerisation of two and above [‡] (-)-epicatechin, (+)-catechin and proanthocyanidins with a degree of polymerisation of 2 to ten.

Table 3: Association between flavan-3-ol intake and risk of CVD and mortality. Hazard ratio and 95% confidence interval[†].

Men (n=11,252)		Q1	Q2	Q3	Q4	Q5	P _{Trend}
CVD Incidence (4403)	Person-Years (all men)	25291	24964	24980	24466	25001	
	Incidents (all men)	800	878	898	952	875	
	Model 1 [‡]	1	0.89 (0.81; 0.98)	0.84 (0.77; 0.93)	0.99 (0.90; 1.09)	0.97 (0.88; 1.07)	0.678
	Model 2 [§]	1	0.87 (0.79; 0.96)	0.81 (0.74; 0.90)	0.91 (0.83; 1.00)	0.90 (0.81; 0.99)	0.176
	- healthy at baseline [#] (n=8636)	1	0.86 (0.76; 0.97)	0.79 (0.70; 0.90)	0.98 (0.86; 1.10)	0.89 (0.78; 1.01)	0.427
	- high risk [¶] (n=8010)	1	0.85 (0.77; 0.95)	0.80 (0.72; 0.89)	0.87 (0.78; 0.97)	0.91 (0.82; 1.02)	0.270
	- at least three days diary (n=10,343)	1	0.85 (0.77; 0.94)	0.82 (0.75; 0.91)	0.91 (0.83; 1.01)	0.91 (0.82; 1.01)	0.329
IHD Incidence (2335)	Person-Years	27351	27293	27371	26872	27413	
	Incidents	425	456	459	531	464	
	Model 1 [‡]	1	0.89 (0.78; 1.02)	0.84 (0.74; 0.96)	1.06 (0.93; 1.20)	0.99 (0.87; 1.13)	0.309
	Model 2 [§]	1	0.85 (0.74; 0.97)	0.81 (0.71; 0.93)	0.94 (0.83; 1.08)	0.88 (0.77; 1.01)	0.338
	- healthy at baseline [#] (n=8636)	1	0.84 (0.70; 1.02)	0.75 (0.62; 0.91)	1.07 (0.89; 1.28)	0.97 (0.80; 1.17)	0.443
	- high risk [¶] (n=8010)	1	0.81 (0.70; 0.93)	0.80 (0.69; 0.92)	0.85 (0.74; 0.98)	0.86 (0.74; 1.00)	0.161
	- at least three days diary (n=10,343)	1	0.85 (0.74; 0.98)	0.85 (0.74; 0.98)	0.97 (0.84; 1.11)	0.94 (0.81; 1.08)	1.000
Stroke Incidence (595)	Person-Years	29575	29515	29613	29686	29614	
	Incidents	89	123	135	117	131	
	Model 1 [‡]	1	0.99 (0.75; 1.30)	0.99 (0.75; 1.29)	0.94 (0.71; 1.24)	1.18 (0.90; 1.54)	0.263
	Model 2 [§]	1	0.99 (0.75; 1.31)	0.98 (0.75; 1.29)	0.88 (0.66; 1.17)	1.08 (0.82; 1.43)	0.727
	- healthy at baseline [#] (n=8636)	1	1.13 (0.78; 1.64)	1.00 (0.68; 1.45)	1.04 (0.71; 1.52)	1.10 (0.75; 1.62)	0.765
	- high risk [¶] (n=8010)	1	0.96 (0.72; 1.30)	0.94 (0.70; 1.26)	0.89 (0.66; 1.20)	1.15 (0.85; 1.54)	0.388
	- at least three days diary (n=10,343)	1	0.87 (0.66; 1.15)	0.86 (0.66; 1.13)	0.79 (0.60; 1.05)	0.95 (0.71; 1.25)	0.670
CVD mortality (1154)	Person-Years	27854	27415	27550	27495	27533	
	Incidents	173	243	264	250	224	
	Model 1 [‡]	1	1.00 (0.80; 1.26)	0.96 (0.77; 1.21)	1.07 (0.85; 1.34)	1.07 (0.85; 1.35)	0.428

Flavan-3-ol intake and CVD risk

Model 2 ^s	1	0.98 (0.78; 1.23)	0.94 (0.75; 1.19)	0.94 (0.75; 1.18)	0.95 (0.74; 1.20)	0.607
- healthy at baseline [#] (n=8636)	1	1.15 (0.79; 1.68)	1.10 (0.76; 1.59)	1.44 (1.00; 2.07)	1.26 (0.86; 1.84)	0.123
- high risk [†] (n=8010)	1	1.04 (0.81; 1.32)	0.95 (0.74; 1.21)	0.96 (0.75; 1.23)	1.00 (0.78; 1.29)	0.852
- at least three days diary (n=10,343)	1	0.91 (0.72; 1.15)	0.90 (0.72; 1.14)	0.93 (0.73; 1.17)	0.93 (0.73; 1.18)	0.677
Person-Years	27854	27415	27550	27495	27533	
Incidents	538	653	690	685	678	
Model 1 [†]	1	0.90 (0.79; 1.04)	0.85 (0.74; 0.97)	0.93 (0.81; 1.06)	1.03 (0.90; 1.18)	0.373
Model 2 ^s	1	0.91 (0.80; 1.05)	0.87 (0.76; 1.00)	0.90 (0.78; 1.03)	0.99 (0.86; 1.14)	0.946
- healthy at baseline [#] (n=8636)	1	1.00 (0.83; 1.21)	0.99 (0.82; 1.19)	1.07 (0.89; 1.28)	1.16 (0.97; 1.40)	0.065
- high risk [†] (n=8010)	1	0.92 (0.79; 1.06)	0.86 (0.74; 1.00)	0.87 (0.75; 1.01)	1.01 (0.86; 1.17)	0.894
- at least three days diary (n=10,343)	1	0.88 (0.76; 1.01)	0.84 (0.73; 0.97)	0.91 (0.79; 1.04)	0.99 (0.86; 1.14)	0.709
Women (n=13,633)						
Person-Years	32794	32236	31938	31954	32524	
Incidents	678	824	905	874	779	
Model 1 [†]	1	0.98 (0.89; 1.09)	1.01 (0.92; 1.12)	1.09 (0.98; 1.21)	1.11 (1.00; 1.23)	0.009
Model 2 ^s	1	0.96 (0.86; 1.06)	0.97 (0.87; 1.07)	1.04 (0.94; 1.16)	1.01 (0.91; 1.13)	0.364
- healthy at baseline [#] (n=10,795)	1	1.06 (0.92; 1.21)	0.97 (0.85; 1.11)	1.04 (0.91; 1.19)	1.12 (0.98; 1.28)	0.138
- high risk [†] (n=8507)	1	0.93 (0.83; 1.04)	0.95 (0.85; 1.06)	1.04 (0.93; 1.16)	0.96 (0.86; 1.08)	0.884
- at least three days diary (n=12,815)	1	0.94 (0.85; 1.05)	0.96 (0.87; 1.06)	1.04 (0.93; 1.15)	1.01 (0.91; 1.13)	0.373
Person-Years	35139	35167	35094	34897	35275	
Incidents	207	269	306	313	230	
Model 1 [†]	1	1.01 (0.84; 1.22)	1.09 (0.91; 1.30)	1.25 (1.05; 1.50)	1.09 (0.91; 1.32)	0.076
Model 2 ^s	1	1.00 (0.83; 1.20)	1.06 (0.88; 1.27)	1.16 (0.97; 1.39)	0.96 (0.80; 1.17)	0.810
- healthy at baseline [#] (n=10,795)	1	1.15 (0.89; 1.49)	0.98 (0.76; 1.27)	1.20 (0.93; 1.54)	1.06 (0.81; 1.38)	0.648
- high risk [†] (n=8507)	1	1.01 (0.83; 1.23)	1.03 (0.85; 1.25)	1.20 (0.99; 1.45)	0.97 (0.78; 1.19)	0.748
- at least three days diary (n=12,815)	1	1.04 (0.86; 1.25)	1.07 (0.89; 1.29)	1.20 (1.00; 1.45)	0.98 (0.80; 1.19)	0.747
Stroke incidence	35935	35994	36089	36022	36005	

Flavan-3-ol intake and CVD risk

(605)	Incidents	96	132	140	129	108	
	Model 1 [†]	1	0.96 (0.73; 1.25)	0.91 (0.70; 1.18)	0.95 (0.73; 1.25)	1.03 (0.78; 1.36)	0.806
	Model 2 [§]	1	0.94 (0.72; 1.23)	0.86 (0.66; 1.12)	0.88 (0.67; 1.16)	0.89 (0.68; 1.19)	0.384
	- healthy at baseline [#] (n=10,795)	1	1.00 (0.69; 1.44)	0.96 (0.67; 1.38)	1.00 (0.69; 1.44)	1.01 (0.69; 1.48)	0.958
	- high risk [‡] (n=8507)	1	0.93 (0.70; 1.24)	0.82 (0.62; 1.09)	0.83 (0.62; 1.11)	0.91 (0.68; 1.23)	0.393
	- at least three days diary (n=12,815)	1	0.91 (0.69; 1.19)	0.86 (0.66; 1.13)	0.87 (0.66; 1.15)	0.91 (0.68; 1.21)	0.502
CVD mortality (833)	Person-Years	3479	34597	34801	34652	35037	
	Incidents	138	173	188	193	141	
	Model 1 [†]	1	0.95 (0.73; 1.25)	0.84 (0.64; 1.11)	0.98 (0.75; 1.28)	0.93 (0.70; 1.25)	0.734
	Model 2 [§]	1	0.92 (0.70; 1.21)	0.78 (0.60; 1.03)	0.89 (0.67; 1.17)	0.81 (0.60; 1.09)	0.166
	- healthy at baseline [#] (n=10,795)	1	1.02 (0.69; 1.51)	0.76 (0.51; 1.13)	0.76 (0.50; 1.15)	0.99 (0.65; 1.50)	0.566
	- high risk [‡] (n=8507)	1	0.95 (0.71; 1.26)	0.76 (0.57; 1.02)	0.88 (0.66; 1.17)	0.88 (0.64; 1.19)	0.322
All cause mortality (2657)	- at least three days diary (n=12,815)	1	0.97 (0.73; 1.28)	0.79 (0.60; 1.05)	0.90 (0.68; 1.20)	0.86 (0.64; 1.16)	0.280
	Person-Years	3479	34597	34801	34652	35037	
	Incidents	437	582	587	586	465	
	Model 1 [†]	1	1.01 (0.86; 1.17)	0.90 (0.77; 1.05)	0.99 (0.85; 1.16)	1.01 (0.85; 1.18)	0.981
	Model 2 [§]	1	1.00 (0.86; 1.18)	0.86 (0.74; 1.01)	0.95 (0.81; 1.12)	0.92 (0.78; 1.09)	0.262
	- healthy at baseline [#] (n=10,795)	1	1.04 (0.85; 1.26)	0.82 (0.67; 1.00)	0.86 (0.70; 1.06)	0.98 (0.79; 1.20)	0.388
Post-menopausal Women (n=8176)	- high risk [‡] (n=8507)	1	1.04 (0.88; 1.24)	0.88 (0.74; 1.05)	0.95 (0.80; 1.14)	1.00 (0.83; 1.20)	0.702
	- at least three days diary (n=12,815)	1	1.02 (0.87; 1.19)	0.87 (0.74; 1.02)	0.94 (0.80; 1.11)	0.93 (0.79; 1.10)	0.254
	Person-Years	18648	18517	18397	18323	18724	
	Incidents	632	644	702	683	645	
	Model 1 [†]	1	0.91 (0.82; 1.02)	0.96 (0.86; 1.07)	1.03 (0.92; 1.15)	1.02 (0.91; 1.14)	0.266
	Model 2 [§]	1	0.89 (0.80; 0.99)	0.92 (0.83; 1.03)	0.99 (0.89; 1.11)	0.94 (0.84; 1.05)	0.793
CVD Incidence (3306)	- healthy at baseline [#] (n=5891)	1	0.94 (0.82; 1.09)	0.87 (0.75; 1.01)	0.93 (0.80; 1.08)	1.00 (0.87; 1.16)	0.976
	- high risk [‡] (n=5998)	1	0.90 (0.80; 1.02)	0.94 (0.84; 1.06)	1.00 (0.89; 1.13)	0.95 (0.84; 1.08)	0.901
	- at least three days diary	1	0.89 (0.80; 1.00)	0.92 (0.83; 1.03)	0.99 (0.88; 1.11)	0.95 (0.84; 1.06)	0.818

Flavan-3-ol intake and CVD risk

		(n=7839)									
		Person-Years	20737	20796	20782	20578	21028				
IHD incidence (1157)	Incidents		218	226	250	259	204				
	Model 1 [†]		1	0.94 (0.78; 1.13)	1.00 (0.84; 1.20)	1.15 (0.96; 1.38)	0.97 (0.80; 1.17)				
	Model 2 [§]		1	0.90 (0.75; 1.09)	0.96 (0.80; 1.16)	1.05 (0.87; 1.26)	0.84 (0.69; 1.02)				
	- healthy at baseline [#] (n=5891)		1	1.05 (0.80; 1.38)	0.92 (0.70; 1.21)	1.10 (0.84; 1.44)	0.94 (0.71; 1.24)				
	- high risk [‡] (n=5998)		1	0.92 (0.75; 1.12)	0.97 (0.79; 1.18)	1.06 (0.87; 1.29)	0.88 (0.72; 1.09)				
	- at least three days diary (n=7839)		1	0.93 (0.76; 1.12)	0.97 (0.80; 1.17)	1.05 (0.87; 1.27)	0.85 (0.70; 1.04)				
Stroke incidence (550)	Person-Years		21517	21509	21576	21538	21685				
	Incidents		110	119	115	107	99				
	Model 1 [†]		1	0.90 (0.69; 1.17)	0.81 (0.62; 1.05)	0.84 (0.64; 1.10)	0.89 (0.67; 1.16)				
	Model 2 [§]		1	0.89 (0.69; 1.16)	0.78 (0.60; 1.02)	0.79 (0.60; 1.04)	0.78 (0.59; 1.03)				
	- healthy at baseline [#] (n=5891)		1	0.92 (0.63; 1.33)	0.88 (0.61; 1.27)	0.87 (0.60; 1.26)	0.88 (0.60; 1.30)				
	- high risk [‡] (n=5998)		1	0.95 (0.71; 1.25)	0.79 (0.59; 1.05)	0.80 (0.60; 1.08)	0.83 (0.61; 1.11)				
CVD mortality (776)	- at least three days diary (n=7839)		1	0.93 (0.71; 1.22)	0.81 (0.62; 1.07)	0.80 (0.61; 1.06)	0.83 (0.62; 1.10)				
	Person-Years		20488	20356	20554	20393	20753				
	Incidents		156	157	156	167	140				
	Model 1 [†]		1	0.94 (0.72; 1.22)	0.77 (0.58; 1.01)	0.92 (0.70; 1.20)	0.89 (0.67; 1.18)				
	Model 2 [§]		1	0.93 (0.72; 1.22)	0.73 (0.56; 0.97)	0.85 (0.65; 1.12)	0.80 (0.60; 1.06)				
	- healthy at baseline [#] (n=5891)		1	1.03 (0.70; 1.52)	0.73 (0.48; 1.09)	0.74 (0.48; 1.12)	0.92 (0.61; 1.39)				
All cause mortality (2329)	- high risk [‡] (n=5998)		1	0.99 (0.74; 1.31)	0.73 (0.54; 0.98)	0.89 (0.66; 1.19)	0.87 (0.64; 1.17)				
	- at least three days diary (n=7839)		1	1.03 (0.78; 1.36)	0.77 (0.58; 1.02)	0.88 (0.66; 1.17)	0.86 (0.64; 1.16)				
	Person-Years		20488	20356	20554	20393	20753				
	Incidents		444	484	477	492	432				
	Model 1 [†]		1	0.97 (0.83; 1.14)	0.85 (0.72; 1.00)	0.96 (0.82; 1.13)	0.97 (0.82; 1.14)				
	Model 2 [§]		1	0.97 (0.83; 1.14)	0.83 (0.70; 0.98)	0.93 (0.79; 1.10)	0.89 (0.76; 1.06)				
	- healthy at baseline [#] (n=5891)		1	1.05 (0.85; 1.28)	0.74 (0.60; 0.92)	0.85 (0.69; 1.05)	0.91 (0.73; 1.13)				
	- high risk [‡] (n=5998)		1	0.99 (0.83; 1.18)	0.86 (0.72; 1.03)	0.95 (0.79; 1.13)	0.95 (0.79; 1.142)				

Flavan-3-ol intake and CVD risk

- at least three days diary (n=7839)	1	1.01 (0.86; 1.19)	0.86 (0.73; 1.02)	0.94 (0.79; 1.11)	0.93 (0.78; 1.10)	0.263
---	---	-------------------	-------------------	-------------------	-------------------	-------

[†] missing values were assumed to be missing at random and were imputed using multiple imputation (n=5) with univariate regression models. [‡]adjusted for BMI and energy intake [§] adjusted for age, BMI, energy intake, systolic blood pressure, plasma cholesterol and vitamin C, intake of fibre, sodium, potassium, fat, saturated fat and alcohol and the following categorical variables: physical activity, smoking status, marital status, education, use of anti-hypertensive drugs and self-reported history of stroke, myocardial infarction and diabetes at baseline, family history of myocardial infarction and – for women – menopausal status and hormone replacement therapy. [#]includes only participants without self-reported history of stroke, myocardial infarction, diabetes mellitus, family history of myocardial infarction and no use of antihypertensive and lipid-lowering drugs. [¶]includes only participants with a 10-year CVD risk 20% higher than normal based on data from the Framingham Heart Study (estimate based Framingham risk score using age, sex, BMI and blood pressure **(36)**).

Figure legends

Figure 1: Study population and exclusion criteria

Figure 2: Relative composition of total dietary flavan-3-ols by flavan-3-ol monomer intake in male participants of EPIC-Norfolk (results for women and post-menopausal women are similar). (-)-Epicatechin and (+)-catechin were the main contributors of total monomer intake in the bottom quintile, while gallated compounds were the main contributor to monomer intake in the top four quintiles. For concomitantly consumed non-monomeric flavanols, similar differences were observed: in the bottom quintile, proanthocyanidins were the dominant class of compounds, while in the other quintiles, theaflavins were more important.

Figure 3: Association between flavan-3-ol intake and risk of CVD in 11,253 men (a) and 13634 women (b) of EPIC-Norfolk. This figure shows HR (95% CI) estimated using the parsimonious model and quintiles of intake of each respective flavan-3-ol group.

Figure 4: Association between flavan-3-ol intake and incidence of CVD in observational epidemiological studies conducted previously. Data shows the estimated HR (95% CI) comparing the bottom and top quintile of intake. Studies included: Zutphen Elderly Study(20); EPIC-Norfolk (this study); HPFS: Health-Professional Follow-Up Study(15); CPS: Cancer Prevention Study II(18); IWH: Iowa Women's Health Study(17); NHS I & II: Nurses Health Study(15). Data for IHD and CHD were combined.

Figure 5: Dietary intervention studies investigating the effect of dietary flavan-3-ols on systolic blood pressure (see Supplemental Table 4 for details) based on (-)-epicatechin intake. Circles indicate studies, which were blinded, conducted for at least 28 days and provided information on (-)-epicatechin based on analyses. All other studies with a duration of 14 days or more are indicated by an X. (-)-epicatechin intake was estimated as 10% of cocoa flavanol intake for studies where no analytical data was available. The histogram shows the distribution of intake in EPIC Norfolk. Error bars were omitted to improve clarity.

Figures

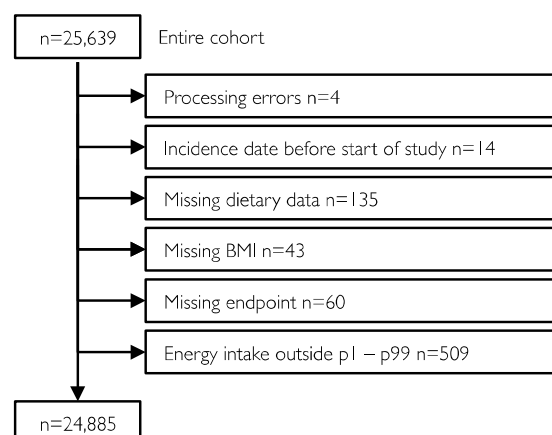


Figure 1: Study population and exclusion criteria

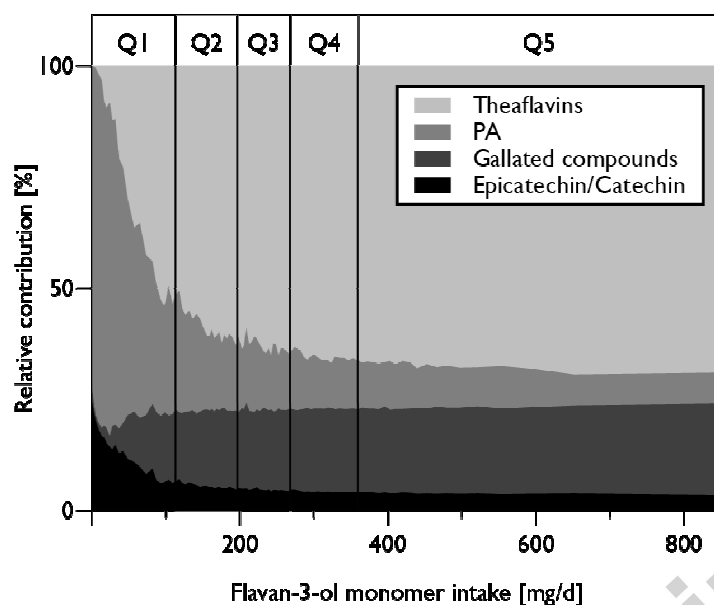


Figure 2: Relative composition of total dietary flavan-3-ols by flavan-3-ol monomer intake in male participants of EPIC-Norfolk (results for women and post-menopausal women are similar). (-)-Epicatechin and (+)-catechin were the main contributors of total monomer intake in the bottom quintile, while gallated compounds were the main contributor to monomer intake in the top four quintiles. For concomitantly consumed non-monomeric flavanols, similar differences were observed: in the bottom quintile, proanthocyanidins were the dominant class of compounds, while in the other quintiles, theaflavins were more important.

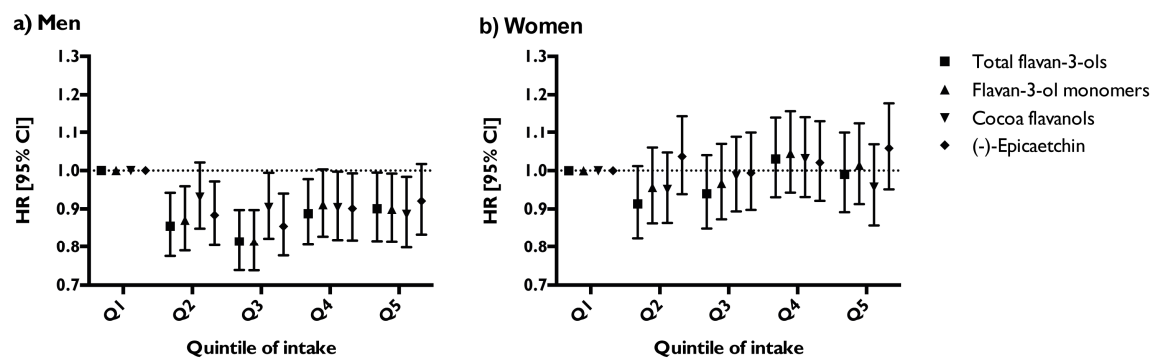


Figure 3: Association between flavan-3-ol intake and risk of CVD in 11,253 men (a) and 13,634 women (b) of EPIC-Norfolk. This figure shows HR (95% CI) estimated using the parsimonious model and quintiles of intake of each respective flavan-3-ol group.

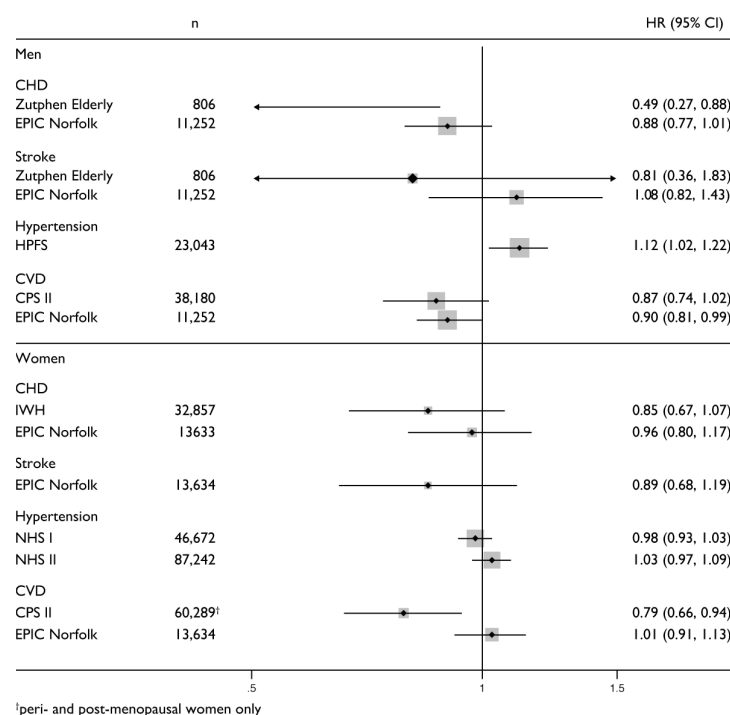


Figure 4: Association between flavan-3-ol intake and incidence of CVD in observational epidemiological studies conducted previously. Data shows the estimated HR (95% CI) comparing the bottom and top quintile of intake. Studies included: Zutphen Elderly Study(20); EPIC-Norfolk (this study); HPFS: Health-Professional Follow-Up Study(15); CPS: Cancer Prevention Study II(18); IWH: Iowa Women's Health Study(17); NHS I & II: Nurses Health Study(15). Data for IHD and CHD were combined.

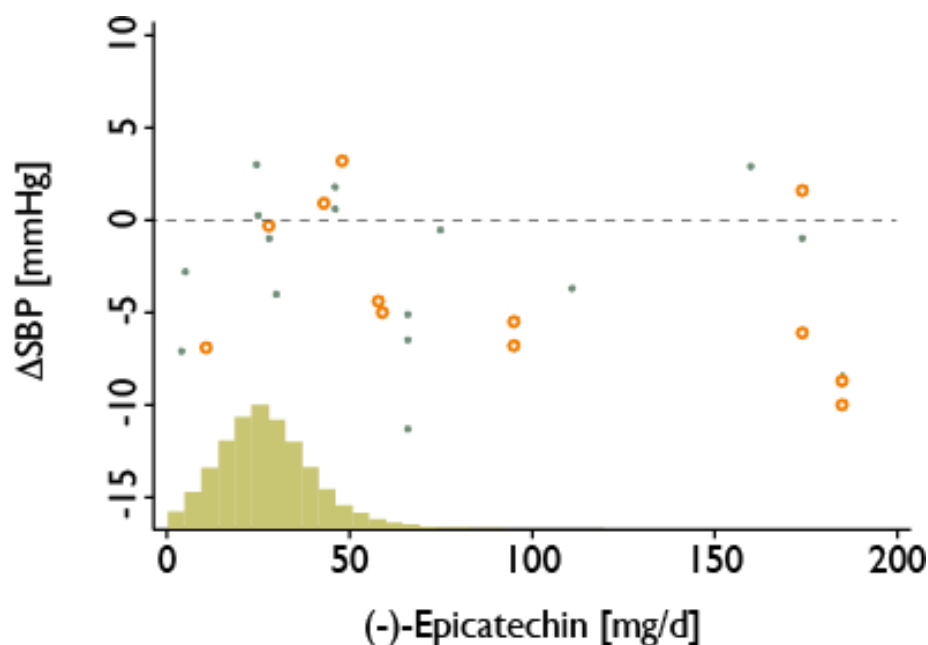


Figure 5: Dietary intervention studies investigating the effect of dietary flavan-3-ols on systolic blood pressure (see Supplemental Table 4 for details) based on (-)-epicatechin intake. Circles indicate studies, which were blinded, conducted for at least 28 days and provided information on (-)-epicatechin based on analyses. All other studies with a duration of 14 days or more are indicated by an X. (-)-epicatechin intake was estimated as 10% of cocoa flavanol intake for studies where no analytical data was available. The histogram shows the distribution of intake in EPIC Norfolk. Error bars were omitted to improve clarity.

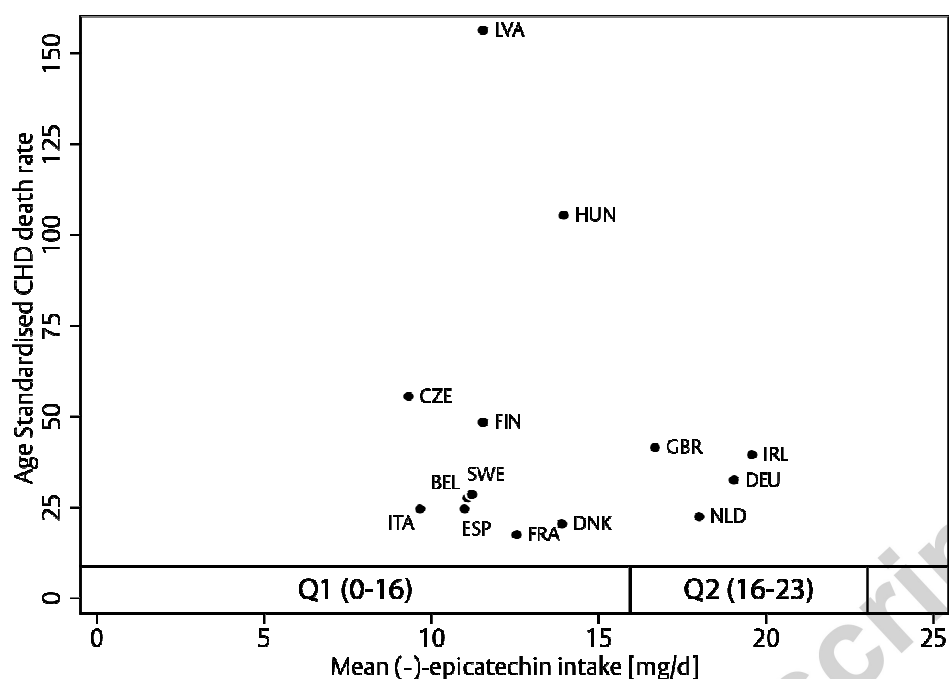


Figure 6: Mean (-)-epicatechin intake (23) and age-standardised CHD mortality (per 100,000, data for 2005) (54) in European countries. (-)-epicatechin intake in EPIC Norfolk is shown for the relevant quintiles.

- Median intake of total flavan-3-ol monomers in EPIC Norfolk was 233 mg/d for men and 217 mg/d for women.
- There were no consistent associations between flavan-3-ol monomer intake and baseline systolic and diastolic blood pressure (BP) in EPIC Norfolk.
- There were no consistent association between flavan-3-ol intake and CVD risk). in EPIC Norfolk
- Flavan-3-ol intake in EPIC-Norfolk is not sufficient to achieve a statistically significant reduction in CVD risk.

