Habitual chocolate consumption and risk of cardiovascular disease among healthy men and women

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Abstract

Objective: The objective of this study is to examine the association between chocolate intake and the risk of future cardiovascular events.

Methods: We conducted a prospective study using data from the European Prospective Investigation into Cancer (EPIC)-Norfolk cohort. Habitual chocolate intake was quantified using the baseline food frequency questionnaire (1993-1997) and cardiovascular end points were ascertained up to March 2008. A systematic review was performed to evaluate chocolate consumption and cardiovascular outcomes.

Results: A total of 20,951 men and women were included in EPIC-Norfolk analysis (mean follow-up 11.3±2.8 years, median 11.9 years). The percentage of participants with coronary heart disease (CHD) in the highest and lowest quintile of chocolate consumption was 9.7% and 13.8% and the respective rates for stroke were 3.1% and 5.4%. The multivariate-adjusted hazard ratio (HR) for CHD was 0.88 (0.77-1.01) for those in the top quintile of chocolate consumption (16-99 g/day) versus non-consumers of chocolate intake. The corresponding HR for stroke and cardiovascular disease (CVD defined by the sum of CHD and stroke) were 0.77 (0.62-0.97) and 0.86 (0.76-0.97). The propensity score matched estimates showed a similar trend. A total of 9 studies with 157,809 participants were included in the meta-analysis. Higher compared to lower chocolate consumption was associated with significantly lower CHD risk (5 studies; pooled RR 0.71 (0.56-0.92)), stroke (5 studies; pooled RR 0.79 (0.70-0.87)), composite cardiovascular adverse outcome (2 studies; pooled RR 0.75 (0.54-1.05)) and cardiovascular mortality (3 studies; pooled RR 0.55 (0.36-0.83)).

Conclusions: Cumulative evidence suggests that higher chocolate intake is associated with a lower risk of future cardiovascular events though residual confounding cannot be excluded.There does not appear to be any evidence to say that chocolate should be avoided in those who are concerned about cardiovascular risk.

Key Questions

What is already known about this subject?

- Chocolate consumption has been associated with a lower risk of cardiovascular disease, but studies have limitations with regard to participant selection and combined outcome measures.

What does this study add?

- We observed evidence that higher intake of chocolate may be associated with lower cardiovascular disease and mortality.

- Our meta-analysis of eight studies found a lower risk of cardiovascular disease with chocolate consumption.

How might this impact clinical practice?

- Our study adds to the previously found inverse associations between chocolate intake and risk of future cardiovascular events.

- There does not appear to be any evidence to say that chocolate should be avoided in those who are concerned about cardiovascular risk.

Introduction

Chocolate is an important dietary source of flavonoid antioxidants, which are hypothesized to have a beneficial effect on endothelial function and protect against cardiovascular disease (CVD) [1]. Evidence from a range of small-scale intervention trials have reported that intake of chocolate resulted in increased high-density lipoprotein (HDL) cholesterol levels, decreased low-density lipoprotein (LDL) oxidation and improved endothelial function [2]. Large-scale intervention studies have not been performed and therefore the potential benefits of raising chocolate consumption on cardiovascular risk are unknown. However, some evidence on the potential beneficial effects of chocolate can be derived from observational studies.

Many studies have evaluated the risk of cardiovascular outcomes with chocolate consumption [1-4]. One meta-analysis which attempted to quantify systematically the effect of high chocolate consumption was limited by inclusion of a heterogeneous spectrum of outcomes including diabetes and heart failure [3]. A more recent meta-analysis specifically evaluated the effects of flavonols (also found in other foods) and the risk of coronary heart disease (CHD) and found no significant association between flavonol intake and CHD [4]. Many cohort studies which examined the association between chocolate and CVD (defined by CHD and stroke in this study) are limited to sex-specific cohorts [5-9]. Two meta-analyses have been performed which pooled many of these studies and found reduced risk of incident stroke [7] and CVD [10] with higher chocolate consumption. However, these studies are limited because in one study only considered stroke while the other study pooled many different cardiovascular events into a single risk estimate.

In order to evaluate the habitual chocolate consumption and the risk of cardiovascular events, we analyzed the data from the European Prospective Investigation into Cancer-Norfolk (EPIC-Norfolk), a large prospective population study in the United Kingdom and

incorporated the results from this observational study into the evidence available to date from the literature by conducting a systematic review and meta-analysis.

Methods

EPIC-Norfolk Cohort study

The European Prospective Investigation into Cancer (EPIC)-Norfolk is a prospective

population study of over 25,000 men and women, resident in Norfolk, United Kingdom

(99.6% white Caucasian). The study methods have been previously described previously in

detail [11]. In brief, at the baseline survey between 1993 and 1997, participants completed a

health and lifestyle questionnaire and provided information on physician-diagnosed chronic

diseases such as cancer, myocardial infarction, stroke and diabetes mellitus. We identified

cigarette smoking habit (never, former and current) [12] and a validated, self-reported

physical activity measure (inactive, moderately inactive, moderately active, active) [13] and

assessed reported intake of alcoholic drinks (where units (8 g alcohol)/week were categorized

into: zero consumers, >0-7, >7-14, >14-21; >21-28; >28 units/week). At the baseline health

examination (N=25,639), a trained nurse measured height, weight and blood pressure

(Acutorr). Cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were

measured in non-fasting blood samples as described previously [11]. Blood samples were

stored at $-80^{°}$C. Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald

formula. Data on lipids were available for 23,074 participants (90%) and for inflammation

markers on minimally 18,643 participants (73%).

Main exposure measure: chocolate consumption

Dietary measurements were obtained by food frequency questionnaire (FFQ), which assessed

overall diet in the past year from 24,782 participants (response rate 97%). Every food item or

group of food items in the questionnaire had nine answer categories: never or less than once

per month, 1-3 per month, once a week, 2-4 per week, 5-6 per week, once a day, 2-3 per day,

4-5 per day, 6+ per day. This instrument and the way it is analysed using the Compositional Analyses from Frequency Estimates (CAFÉ), are described in detail elsewhere [14]. Three questions from the FFQ were considered indicative of chocolate consumption, namely "Chocolates singles or squares" (average portion size of 8 g), "Chocolate snack bars, e.g. Mars, Crunchie" (average portion size of 50 g) and "Cocoa, hot chocolate (cup)" (average portion size of 12 g powder weight, the liquid to make up the beverage was hence not included). Frequency categories were multiplied by the portion size to derive the amount of chocolate product eaten (g/day). The sum of the weights of these food items consumed, rather than their flavonoid or cocoa content, formed the measure of exposure.

Clinical outcomes

Participants admitted to hospital were identified using their unique National Health Service number by data linkage with ENCORE (East Norfolk Health Authority database). All participants were flagged for death certification at the UK Office of National Statistics, ascertaining vital status for the entire cohort. CHD was defined as ICD10 codes I20-25 (which includes the spectrum of CHD including myocardial infarction, unstable angina and stable angina); stroke was defined as ICD10 codes I60-69 (which includes ischemic stroke, hemorrhagic stroke and stroke of undetermined cause). CVD was defined as the combination of CHD and stroke. A previous validation study in our cohort indicated high specificity for such case ascertainment [15]. We report data with follow-up up to 31 March 2008, an average of 11.3 years (2.8 standard deviation, median of 11.9 years). The study was approved by the Norwich District Health Authority Ethics Committee. All participants provided signed informed consent.

Statistical analysis

Participants without a completed FFQ (n=857), with prevalent myocardial infarction or stroke (n=1102) and whose reported chocolate intake was more than 5 standard deviations above the median (i.e. 100 g/day) were excluded from the analyses (n=86). Leaving 23,638 participants eligible; we further excluded 2687 participant who missed data on one or more covariables (n=20,951). Baseline characteristics were calculated by quintiles of chocolate consumption. Since 4,195 participants (approximately 20%) reported zero chocolate intake, the lowest quintile corresponds to the non-users only and was used as the reference category in Cox proportional hazards model. Hazard ratios (HR) and corresponding 95% confidence intervals (95%CI) for the risk of future CHD, stroke or CVD were calculated using four models. Regression model 1 adjusted for sex and age. Regression model 2 additionally adjusted for lifestyle factors: smoking, physical activity, energy intake (per MJ/d) and alcohol intake (categorical). Regression model 3 adjusted for the variables in model 2 and possible mediators: body mass index, systolic blood pressure, LDL cholesterol, HDL cholesterol and prevalent diabetes. In model 4 we additionally adjusted for C-reactive protein (CRP). Analyses were performed using SPSS version 17.0 (Chicago, Illinois, USA).

Systematic review and meta-analysis

To be eligible for inclusion in our review, studies had to report on the association between chocolate consumption and cardiovascular outcomes. We searched PubMed and EMBASE from inception until June 2013 using the terms described in **Data Supplement 1**, with no language limitations, and we checked bibliographies of included articles. In addition to our search of EMBASE (which already includes unpublished abstracts and conference reports) we also searched conference abstracts from the European Society of Cardiology Congresses from 2005-2013 inclusive. A further search of ISI Web of Science was conducted in February 2014

to identify additional articles. Two reviewers independently screened abstracts and titles, and then obtained full-text versions of potentially relevant studies to confirm eligibility. Data extraction of included studies was performed by CSK as well as JKY, and checked by YKL. Study validity was evaluated based on ascertainment of chocolate consumption and cardiovascular outcomes as well as steps taken to reduce confounding in the primary studies. We pooled data using the inverse variance method and random effects model in RevMan 5.2 software (Nordic Cochrane Center, Copenhagen, Denmark). For these comparisons, we used the multivariable adjusted measures of association (hazard ratios, relative risks or odds ratios) for the highest category of chocolate consumption versus the lowest category of consumption. Heterogeneity was estimated using I^2, and we considered a value greater than 50% to demonstrate substantial heterogeneity [16]. We planned to evaluate publication bias through asymmetry testing if there were >10 studies in the dataset, and no evidence of significant heterogeneity [17].

Sensitivity Analyses

We performed additional analysis to determine if a similar direction of effect was observed using propensity score matching on the pre-specified covariates of models 1, 2 and 3. Propensity score matching were performed in STATA using the teffects psmatch function which estimates treatment effects from observational data [18]. The results of the propensity score analysis was used as sensitivity analysis for the meta-analysis. We conducted additional analysis excluding the Djousse study because it was a cross-sectional study.

Results

EPIC-Norfolk cohort

A complete dataset on relevant baseline characteristics were available for 20,951 study participants; 9,214 men and 11,737 women (n=16,162 when restricted to participants with CRP data). Mean follow-up was 11.9 ± 2.8 years, total person year 236,942 years. A total of 3,013 (14.4%) people experienced a fatal or non-fatal CHD event, stroke or both, referred to as CVD. Among these participants with CVD events, 2,434 (11.6%) had a CHD event and 848 (4.0%) had a stroke event. When analyses were limited to people with available CRP at baseline, a total of 2,207 (13.7%) study participants experienced a CVD event while 1,754 (10.9%) and 648 (4.0%) had a CHD event and/or stroke event, respectively.

Chocolate consumption in the EPIC-Norfolk cohort

The median daily chocolate consumption was 4.6 g/day (interquartile range 0.6 to 12.0); among consumers only, median chocolate intake was 7.0 g/day (interquartile range 3.5 to 15.5). Higher chocolate consumption was associated with trends towards a beneficial cardiovascular risk factor profile including lower age, lower body mass index, lower waist/hip ratio, lower systolic blood pressure, lower levels of apolipoprotein B (apoB) and CRP, a lower prevalence of diabetes mellitus and more physical inactivity (**Table 1**). In contrast, higher chocolate consumption was more prevalent among men and among current smokers. Higher chocolate intake was associated with a higher energy intake, with lower contributions from protein and alcohol sources and higher contribution from fat and carbohydrates.

Chocolate consumption and risk of cardiovascular disease in EPIC-Norfolk

Higher chocolate intake was associated with a statistically significant lower risk of CVD, with stronger associations for CVD mortality than for total CVD (hospitalization or mortality)

(**Table 2**). HRs attenuated after adjustment, but remained borderline significant for total
CVD (HR 0.89 (95% CI 0.79-1.00)) and CVD mortality (HR 0.75 (95%CI 0.62-0.92)).
Adjustment for CRP minimally changed the effect estimates. A significant dose-response
association was present for both total incidence and CVD mortality. We assessed the
proportionality assumption graphically and the results suggest that proportional hazards were
maintained with time.

Higher chocolate consumption was associated with a lower risk of hospitalization or mortality
due to CHD in crude and minimally adjusted models (**Table 3**). This association was
attenuated after adjustment for a range of cardiovascular risk factors and after additional
adjustment for a set of dietary parameters (HR 0.91, 95%CI 0.80-1.04). In the smaller sample
of participants with CRP data, we observed a significant association with 18% lower risk in
quintile 5 versus quintile 1 (95%CI 0.70-0.97). Participants with a high chocolate intake also
had a lower risk of stroke. The sex- and age- adjusted HR was 0.77 (95%CI 0.62-0.96) for top
versus bottom quintile of chocolate consumption (**Table 3**). This association remained
statistically significant upon adjustment for smoking, physical activity and dietary variables.
Additional adjustment for mediators did not materially change the estimate (HR 0.78, 95%CI
0.63-0.98). Analyses using mortality as outcome rather than combined mortality and
hospitalizations, showed lower risk estimates for CVD, CHD and stroke, although the number
of events were substantially lower and therefore the confidence intervals became wider.

Propensity score matched analysis for chocolate consumption and risk of cardiovascular disease

The baseline characteristics of the unmatched and propensity matched cohorts are shown in **Appendix Table 1**. A Love plot was used to examine the standardized difference in covariates before and after propensity score adjustment (**Appendix Figure 1**). The risk of CHD and stroke by quintiles of chocolate intake considering various levels of adjustments and propensity score matching are shown in **Appendix Table 2**. After propensity score matching, a trend for benefit with chocolate consumption was apparent for both CHD and stroke, but with lower sample sizes and wider confidence intervals they were not statistically significant.

Systematic Review and Meta-Analysis

Search results and studies of chocolate and cardiovascular disease

We screened 392 titles and abstracts and identified 8 studies [5-9,19-21] that met eligibility criteria (**Appendix Figure 2**). Including the EPIC-Norfolk study, there were a total of 9 studies with 157,809 participants included in the meta-analysis. These studies included 7 cohort studies, 1 post-hoc analysis of a randomized trial and 1 cross-sectional study and the follow up duration of the cohort studies ranged from 8 to 16 years (**Table 4**Table 4). Two studies were conducted in the United States and one study was conducted in Australia but the remainder were conducted in Europe (United Kingdom, Netherlands, Germany and Sweden). Three studies included both men and women but the remainder were sex specific (two studies of men and two studies of women). The mean age of the participants in the included studies ranged from 49 to 79 years.

Bias assessment for studies of chocolate and cardiovascular disease

Different methods for evaluating and ascertaining chocolate consumption and cardiovascular outcomes were used across the studies (**Appendix Table 3**). One study used patient interviews to ascertain chocolate consumption but the remaining studies used questionnaires. The majority of studies used ICD codes to ascertain cardiovascular diagnoses. Five of the studies used linkage of data to mortality registry/records in order to ascertain mortality. All the included studies were able to use a variety of adjustments to account for the effect of confounders; for the meta-analysis, we used data from Model 2 of EPIC-Norfolk. While a key cardiovascular risk factor such as body mass index was adjusted for in seven studies, other important risk factors such as cholesterol were only considered in two studies (**Appendix Table 4**). We considered the cross-sectional study to have lower validity due to the inability to draw a temporal relationship between chocolate use and adverse cardiovascular outcomes.

Chocolate consumption and risk of cardiovascular events

Overall, chocolate consumption was significantly associated with lower risk of CHD across 5 studies, (pooled RR 0.71 95% CI 0.56-0.92, I^2=61%) (**Figure 1**). The risk of CHD mortality from one study showed no significant difference with and without chocolate consumption (RR 0.98 95% CI 0.88-1.10). For risk of stroke with chocolate consumption there was significantly lower risk of both stroke incidence (pooled RR 0.79 95% CI 0.70-0.87, I^2=0%, 5 studies) (**Figure 1)** and mortality (RR 0.85 95% CI 0.74-0.98, 1 study). There was a significant lower risk of any cardiovascular event (pooled RR 0.75 95% CI 0.54-1.05, I^2=70%, 2 studies) and cardiovascular mortality (pooled RR 0.55 95% CI 0.36-0.83, I^2=67%, 3 studies) (**Figure 2**). The propensity matched results were incorporated in the meta-analysis and this continued to show a significant association between chocolate consumption and reduction in CHD (pooled RR 0.72, 95% CI 0.55-0.93, I^2=63, 5 studies) and stroke (pooled

RR 0.78, 95% CI 0.70-0.88, I^2=0%, 5 studies) (**Figure 3**). Publication bias was not assessed because there were fewer than 10 studies included in the analysis.

We conducted additional analysis excluding the Djousse study because it was a cross-sectional study. The lower risk associated with chocolate and CHD was slightly less after exclusion of this study but still remained statistically significant (RR 0.84 95% CI 0.75-0.95 vs. 0.79 95% CI 0.70-0.87) (Data not shown).

Discussion

In this large prospective population study, higher intake of chocolate up to 100 g/day was associated with a lower risk of CVD and stroke, with stronger associations for mortality than total incidence. We have built on the findings of the previous meta-analysis [7,10] by including this current study in an updated meta-analysis. The reviews by Larsson et al [7] only considered stroke as an outcome while the review by Zhang et al [10] considered CVD. The current review includes stroke, stroke mortality, CHD and CHD mortality separately. In addition, we included the EPIC-Norfolk cohort which is a Caucasian population. Our results indicate that chocolate consumption was associated with a lower risk of CVD.

A number of issues have to be taken into account when interpreting the results of the present study. Although FFQs are well-established methods to quantify dietary information in large-scale population studies, they have limitations. Importantly, recall bias as well as underreporting, particularly among women and participants who are obese [22]. Underreporting is selective, and includes sweets and snacks. It is possible that lower CVD rates among people who report consuming more chocolate might be due to differential underreporting of chocolate intake in those with potentially greater CVD risk such as the obese and physically inactive. It has been suggested that dark chocolate may have more beneficial effects than milk chocolate [23]. Milk chocolate is more frequently consumed than dark chocolate in this cohort [unpublished results]; however, we still observed a reduced risk of CVD. This may indicate that not only flavonoids, but also other compounds -possibly related to milk constituents such as calcium and fatty acids- may form an explanation of the observed association. In addition, the whole dietary pattern may be of relevance, giving concern for unmeasured confounding. Therefore, this observational study cannot provide evidence on the potential causality of the observed association.

We used the Food Frequency Questionnaire (FFQ) to assess chocolate intake and this measure of dietary assessment method has been validated in the EPIC-Norfolk cohort [24]. While the FFQ is prone to recall bias and requires the participant to 'average out' over a long period of time, this method has the advantage that it covers a longer time-frame than other methods such as 7-day diet diary (7dDD) and 24h-Diet Recall. Chocolate consumption might be a more episodically consumed food for a proportion of the participants and this could have miss-classified the participant as a non-consumer or a high consumer depending in which week the 7dDD or day of 24h-Diet Recall would have been completed.

It is possible that part of the observed association could be explained by reverse causality in that people with a higher risk profile, including those with obesity, diabetes mellitus, or prevalent CVD, eat less chocolate-containing foods than people who have a perceived healthy risk profile. However, we excluded people who reported prevalent CVD at the baseline health questionnaire, and analyses were adjusted for diabetes mellitus. We observed that the reference group (non-chocolate consumers) had the highest mean BMI, highest median CRP, highest proportion of participants with diabetes, highest levels of inactivity and lowest fat intake compared to the other quintiles of chocolate consumers. Alternatively it may be that higher chocolate consumers have other behaviours that are beneficial for cardiovascular health. Participants with a higher energy intake are also likely to have a higher energy expenditure due to physical activity. They are hence more likely to consume more foods, including chocolate containing foods, which may explain part of the observed associations, although stratified analyses suggested that the association found was homogeneous across levels of physical activity. In addition to multivariable adjustment, we conducted stratified analyses and propensity score matching for baseline differences. Nevertheless we cannot exclude residual confounding from these or other unmeasured factors. The consumption of

chocolate as well as its influence on cardiovascular risk may be different depending on ethnicity of participants. External validity of our results may be limited to Caucasians.

Our results are somewhat surprising since the expectation was that benefits of chocolate consumption would be mainly associated with dark chocolate rather than the commercially available products generally used in a British population which are high in sugar content and fat. One study has shown reduced incidence of diabetes among men and women with chocolate intake [25], and other studies have shown that chocolate consumption increases body weight [26,27]. This may suggest that there is a balance between benefit and risk with chocolate intake which is dependent on the risk profile of the individual including baseline weight and dose of chocolate intake.

We did not include heart failure as an outcome for the current analysis for a few reasons. The pathophysiology of CHD and stroke which relates to atherosclerosis differs from that of heart failure. The physiological changes in heart failure are largely driven by neuroendocrine dysregulation and renin-angiotensin-aldosterone system activation. The mechanism by which chocolate might impact on these pathways may differ. In addition, the risk factors and confounders in heart failure differ from those in the cardiovascular disease analysis.

This analysis included clinical biomarkers and anthropometry measured by trained staff. Adjustment for these variables attenuated the association between chocolate consumption and risk of CVD, CHD and stroke, but particularly in case of stroke and fatal CVD, associations remained significant. CRP was available for a subcohort, in which we observed a better cardiovascular risk profile; however, we observed that CRP did not modify the association between chocolate and CVD, CHD or stroke. Pathways other than anti-inflammatory

markers, such as blood pressure, might be a more likely mechanism to explain the observed association between chocolate and CVD/stroke.

Bias may also affect the systematic review if the original studies made post-hoc decisions on particular outcomes or categories for analysis based on nature of the findings. The primary studies collected data using different categories of chocolate consumption (e.g. frequent vs. rare, different quintiles or quartiles) and it seemed that some of the studies made post-hoc decisions on what the cut-offs were in defining categories for analysis (including pooling certain categories while excluding others). This raises the possibility of bias from selective outcome and analysis reporting where categorical cut-offs could have been chosen based on statistical significance of findings. Nevertheless, the cumulative evidence reported in this study suggests that high chocolate consumption may be associated with cardiovascular benefit.

Future research is still needed to explore the association between chocolate and CVD. Studies are needed to better understand the biological mechanism by which chocolate and flavonoids reduce the risk of CVD. It is also possible that some individuals will not benefit from increased chocolate consumption such as those who are overweight or diabetic. More research is needed to identify individuals who would benefit the most.

In conclusion, our findings support the previously reported association between habitual chocolate intake and a lower risk of CHD events in the large EPIC-Norfolk prospective population study and we have further set this in context with consistent results seen in meta-analysis of current evidence. While randomized controlled trials of chocolate and cardiovascular endpoints could be conducted, feasibility is uncertain. For the time being,

within the general context of existing recommendations for behaviours conducive to cardiovascular health, there does not appear to be evidence that chocolate should be avoided in terms of impact on cardiovascular risk.

Competing Interests

The authors have no competing interests to declare.

Contributors

KTK and NJW are Principal Investigators of EPIC-Norfolk cohort. SMB analyzed the EPIC-Norfolk data. RN is responsible for data management and linkage and contributed to data analysis. MAHL and SMB designed the cohort analysis. CSK and YKL performed literature search for systematic review. CSK and JKY screened and collected the data for systematic review which was checked by YKL and PKM. CSK and YKL conducted the meta-analysis. SMB, PKM, CSK and YKL drafted the paper and all authors contributed to the writing of the paper. SMB and CSK are joint first authors. PKM and KTK are joint last authors.

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Supplementary Table 2: Hazard ratios of coronary heart disease and stroke by quintiles of chocolate intake in 20,951 men and women of EPIC-Norfolk using propensity score analyses

Supplementary Table 3: Quality assessment of studies which evaluated chocolate consumption and cardiovascular disease.

Supplementary Table 4: Use of adjustments or exclusions in studies which evaluated chocolate consumption and cardiovascular disease.

Data Supplement 2: PRISMA and STROBE statement

1   **Table 1: Baseline cardiovascular risk factors by quintiles of chocolate intake in 20,951 men and women of EPIC-Norfolk.**

Quintiles of daily chocolate intake	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	P	Total
Range (g/d)	0	0.6-3.5	4.1-7.0	7.2-15.5	15.6-98.8		
Median (interquartile range) (g/d)							
Chocolate intake	0	1.1 (0.6,3.4)	4.6 (4.1,6.3)	9.2 (8.1,12.0)	24.9 (22.1,39.5)		
Chocolate singles	0	0.6 (0.0,1.1)	0.6 (0.6,1.1)	1.1 (0.6,3.4)	1.1 (0.6,3.4)		
Chocolate bars	0	0.0 (0.0,0.0)	3.5 (3.5,3.5)	7.0 (0.0,7.0)	21.5 (21.5,21.5)		
Chocolate beverage	0	0.0 (0.0,0.0)	0.0 (0.0,0.8)	0.8 (0.0,5.2)	0.0 (0.0,5.2)		
N	4195	4437	4035	4106	4178		20,951
Sex, men	44.1 (1852)	43.4 (1927)	40.8 (1648)	43.5 (1786)	47.9 (2001)	< 0.001	44.0 (9214)
Age, years	61 ± 9	60 ± 9	58 ± 9	59 ± 9	57 ± 9	< 0.001	59 ± 9
Body mass index, kg/m2	26.3 ± 4.1	26.1 ± 3.8	26.3 ± 3.7	26.2 ± 3.8	26.0 ± 3.7	< 0.001	26.2 ± 3.8
Waist/hip ratio*	0.86 ± 0.09	0.85 ± 0.09	0.84 ± 0.09	0.85 ± 0.09	0.85 ± 0.09	< 0.001	0.85 ± 0.09
Smoking - current	10.5 (440)	10.7 (473)	11.4 (459)	11.4 (469)	13.0 (543)	< 0.001	11.4 (2384)
- previous	44.0 (1847)	43.1 (1914)	40.1 (1618)	40.9 (1678)	38.5 (1610)		41.4 (8667)
- never	45.5 (1908)	46.2 (2050)	48.5 (1958)	47.7 (1959)	48.5 (2025)		47.3 (9900)
Diabetes mellitus	4.6 (193)	1.6 (73)	0.9 (38)	0.9 (38)	0.7 (29)	< 0.001	1.8 (371)

Systolic blood pressure, mmHg	137 ± 19	136 ± 18	134 ± 18	134 ± 18	135 ± 17	< 0.001	135 ± 18
Diastolic blood pressure, mmHg	83 ± 11	83 ± 11	82 ± 11	82 ± 11	82 ± 11	0.010	82 ± 11
Total cholesterol, mmol/l	6.2 ± 1.1	6.2 ± 1.1	6.1 ± 1.1	6.1 ± 1.1	6.1 ± 1.1	0.001	6.1 ± 1.1
LDL-cholesterol, mmol/l	4.0 ± 1.0	4.0 ± 1.0	3.9 ± 1.0	4.0 ± 1.0	3.9 ± 1.0	0.07	4.0 ± 1.0
HDL-cholesterol, mmol/l	1.43 ± 0.43	1.43 ± 0.42	1.44 ± 0.42	1.43 ± 0.41	1.40 ± 0.41	< 0.001	1.43 ± 0.42
Triglycerides, mmol/l	1.5 (1.1-2.2)	1.5 (1.1-2.1)	1.5 (1.0-2.1)	1.5 (1.1-2.1)	1.5 (1.1-2.1)	0.20	1.5 (1.1-2.1)
Apolipoprotein AI, mg/dl*	156 ± 35	157 ± 35	157 ± 35	156 ± 34	155 ± 36	0.29	156 ± 35
Apolipoprotein B, mg/dl*	97 ± 26	97 ± 25	96 ± 25	96 ± 26	94 ± 25	0.001	96 ± 26
C-reactive protein, mg/l*	1.6 (0.8-3.2)	1.5 (0.7-3.2)	1.5 (0.7-3.2)	1.4 (0.7-3.1)	1.4 (0.7-2.9)	< 0.001	1.5 (0.7-3.1)
Physical activity - Inactive	32.7 (1373)	28.6 (1267)	26.9 (1084)	27.6 (1134)	27.2 (1138)	< 0.001	28.6 (5996)
- Moderately inactive	27.5 (1153)	30.2 (1340)	29.4 (1188)	30.2 (1241)	28.1 (1174)		29.1 (6096)
- Moderately active	21.8 (914)	23.7 (1051)	24.1 (971)	22.8 (936)	24.3 (1015)		23.3 (4887)
- Active	18.0 (755)	17.6 (779)	19.6 (792)	19.4 (795)	20.4 (851)		19.0 (3972)
Energy intake by FFQ, kJ/day	7754 ± 2264	8043 ± 2233	8359 ± 2311	8934 ± 2411	9854 ± 2664	< 0.001	8584 ± 2494
Protein intake by FFQ, %en	17.4 ± 3.4	17.3 ± 3.2	17.1 ± 3.0	16.7 ± 2.9	15.5 ± 2.7	< 0.001	16.9 ± 3.2
Fat intake by FFQ, %en	32.8 ± 6.7	33.8 ± 6.2	34.3 ± 6.1	34.7 ± 5.7	35.8 ± 5.4	< 0.001	34.3 ± 6.1
Carbohydrate intake by FFQ, %en	47.8 ± 7.0	47.3 ± 6.3	47.3 ± 6.1	47.8 ± 5.7	48.0 ± 5.5	< 0.001	47.7 ± 6.2

Alcohol intake by FFQ, %en	3.3 ± 5.3	3.4 ± 4.7	3.1 ± 4.2	2.7 ± 3.6	2.5 ± 3.5	< 0.001	3.0 ± 4.3
Alcohol intake by HLQ, units/week	3 (1-10)	4 (2-11)	4 (2-10)	4 (1-9)	4 (1-10)	< 0.001	4 (1-10)

2 Data are presented as mean ± standard deviation, percentage (number) or median (interquartile range). Differences between groups were analysed by ANOVA

3 for continuous variables, and by Chi-square test for categorical variables. Continuous variables with a skewed distribution (triglycerides, C-reactive protein,

4 alcohol intake) were analyzed by Kruskal-Wallis test.

5 * These variables have missing data: $N_{waist/hip\ ratio}$= 20,928; N_{ApoAI}=16,035; N_{ApoB}=16022; N_{CRP}=16,162.

6

Table 2: Risk of total (fatal and non-fatal) and fatal cardiovascular disease incidence by quintiles of chocolate intake in EPIC-Norfolk (1993-2008).

Range (g/d)	Total	Quintile 1 0	Quintile 2 0.6-3.5	Quintile 3 4.1-7.0	Quintile 4 7.2-15.5	Quintile 5 15.6-98.8	P linearity
Total CVD/n (%)	3013/20,951 (14.4)	729/4195 (17.4)	693/4437 (15.6)	528/4035 (13.1)	563/4106 (13.7)	500/4178 (12.0)	
Model 1		1.00	0.94 (0.85-1.05)	0.94 (0.84-1.05)	0.87 (0.78-0.98)	0.84 (0.75-0.94)	0.001
Model 2		1.00	0.98 (0.88-1.08)	0.97 (0.87-1.09)	0.90 (0.80-1.00)	0.86 (0.76-0.97)	0.006
Model 3		1.00	0.99 (0.89-1.10)	0.99 (0.88-1.10)	0.91 (0.81-1.02)	0.89 (0.79-1.00)	0.018
	2207/16,162 (13.7)	548/3251 (16.9)	512/3406 (15.0)	375/3102 (12.1)	422/3220 (13.1)	350/3183 (11.0)	
Model 4a		1.00	1.00 (0.89-1.13)	0.96 (0.84-1.09)	0.90 (0.79-1.03)	0.82 (0.72-0.95)	0.003
Model 4b		1.00	1.00 (0.88-1.12)	0.95 (0.83-1.09)	0.90 (0.79-1.02)	0.82 (0.71-0.95)	0.003
Fatal CVD/n (%)	1107/20,951 (5.3)	308/4195 (7.3)	238/4437 (5.4)	193/4035 (4.8)	206/4106 (5.0)	162/4178 (3.9)	
Model 1		1.00	0.77 (0.65-0.92)	0.90 (0.75-1.07)	0.78 (0.66-0.94)	0.71 (0.59-0.86)	0.001
Model 2		1.00	0.80 (0.68-0.95)	0.94 (0.79-1.13)	0.81 (0.67-0.97)	0.73 (0.60-0.89)	0.005
Model 3		1.00	0.82 (0.69-0.98)	0.96 (0.80-1.15)	0.82 (0.68-0.98)	0.75 (0.62-0.92)	0.011
	805/16,162 (5.0)	231/3251 (7.1)	174/3406 (5.1)	133/3102 (4.3)	154/3220 (4.8)	113/3183 (3.6)	
Model 4a		1.00	0.82 (0.67-1.00)	0.91 (0.73-1.13)	0.81 (0.66-1.00)	0.71 (0.56-0.89)	0.008

Model 4b	1.00	0.81 (0.66-0.99)	0.90 (0.73-1.12)	0.80 (0.65-0.99)	0.70 (0.55-0.89)	0.008

9   Model 1 adjusted for sex and age.
10 Model 2 adjusted for sex, age, smoking, physical activity, energy intake and alcohol consumption.
11 Model 3: as model 2 and diabetes, body mass index, systolic blood pressure, LDL-cholesterol and HDL-cholesterol.
12 Model 4a: as model 3, but restricted to number of participants for whom CRP is available (N=16,162).
13 Model 4b: as model 3 and C-reactive protein (N=16,162).
14

15 **Table 3: Risk of total (fatal and non-fatal) coronary heart disease and stroke incidence by quintiles of chocolate intake in EPIC-Norfolk**
16 **(1993-2008).**

	Total	Quintile 1 0	Quintile 2 0.6-3.5	Quintile 3 4.1-7.0	Quintile 4 7.2-15.5	Quintile 5 15.6-98.8	P linearity
Range (g/d)							
CHD/n (%)	2434/20,951 (11.6)	577/4195 (13.8)	564/4437 (12.7)	437/4035 (10.8)	449/4106 (10.9)	407/4178 (9.7)	
model 1		1.00	0.97 (0.86-1.09)	0.97 (0.86-1.10)	0.87 (0.77-0.99)	0.85 (0.75-0.96)	0.003
model 2		1.00	1.01 (0.90-1.13)	1.01 (0.89-1.15)	0.91 (0.80-1.03)	0.88 (0.77-1.01)	0.024
model 3		1.00	1.03 (0.91-1.15)	1.03 (0.91-1.17)	0.92 (0.81-1.05)	0.91 (0.80-1.04)	0.06
	1754/16,162 (10.9)	435/3251 (13.4)	407/3406 (11.9)	307/3102 (9.9)	325/3220 (10.1)	280/3183 (8.8)	
model 4a		1.00	1.00 (0.87- 1.15)	0.98 (0.85- 1.14)	0.88 (0.76- 1.01)	0.83 (0.71- 0.97)	0.006
model 4b		1.00	1.00 (0.87- 1.14)	0.98 (0.84- 1.13)	0.87 (0.75- 1.01)	0.82 (0.70- 0.97)	0.005
Stroke/n (%)	848/20,951 (4.0)	226/4195 (5.4)	187/4437 (4.2)	139/4035 (3.4)	165/4106 (4.0)	131/4178 (3.1)	
model 1		1.00	0.83 (0.68-1.00)	0.84 (0.68-1.04)	0.85 (0.70-1.04)	0.77 (0.62-0.96)	0.036
model 2		1.00	0.85 (0.70-1.03)	0.87 (0.70-1.07)	0.86 (0.70-1.06)	0.77 (0.62-0.97)	0.046
model 3		1.00	0.86 (0.71-1.05)	0.87 (0.71-1.08)	0.88 (0.71-1.07)	0.78 (0.63-0.98)	0.06
	648/16,162 (4.0)	168/3251 (5.2)	149/3406 (4.4)	100/3102 (3.2)	132/3220 (4.1)	99/3183 (3.1)	
model 4a		1.00	0.95 (0.76-1.18)	0.87 (0.67-1.12)	0.94 (0.74-1.18)	0.81 (0.62-1.05)	0.14

model 4b	1.00	0.95 (0.76-1.18)	0.87 (0.67-1.11)	0.94 (0.74-1.18)	0.80 (0.62-1.05) 0.14

17 Model 1 adjusted for sex and age.
18 Model 2 adjusted for sex, age, smoking, physical activity, energy intake and alcohol consumption.
19 Model 3: as model 2 and diabetes, body mass index, systolic blood pressure, LDL-cholesterol and HDL-cholesterol.
20 Model 4a: as model 3, but restricted to number of participants for whom CRP is available (N=16,162).
21 Model 4b: as model 3 and C-reactive protein (N=16,162).
22

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25 **Table 4: Study design, participants, follow up and outcomes for studies evaluating chocolate consumption and cardiovascular disease.**

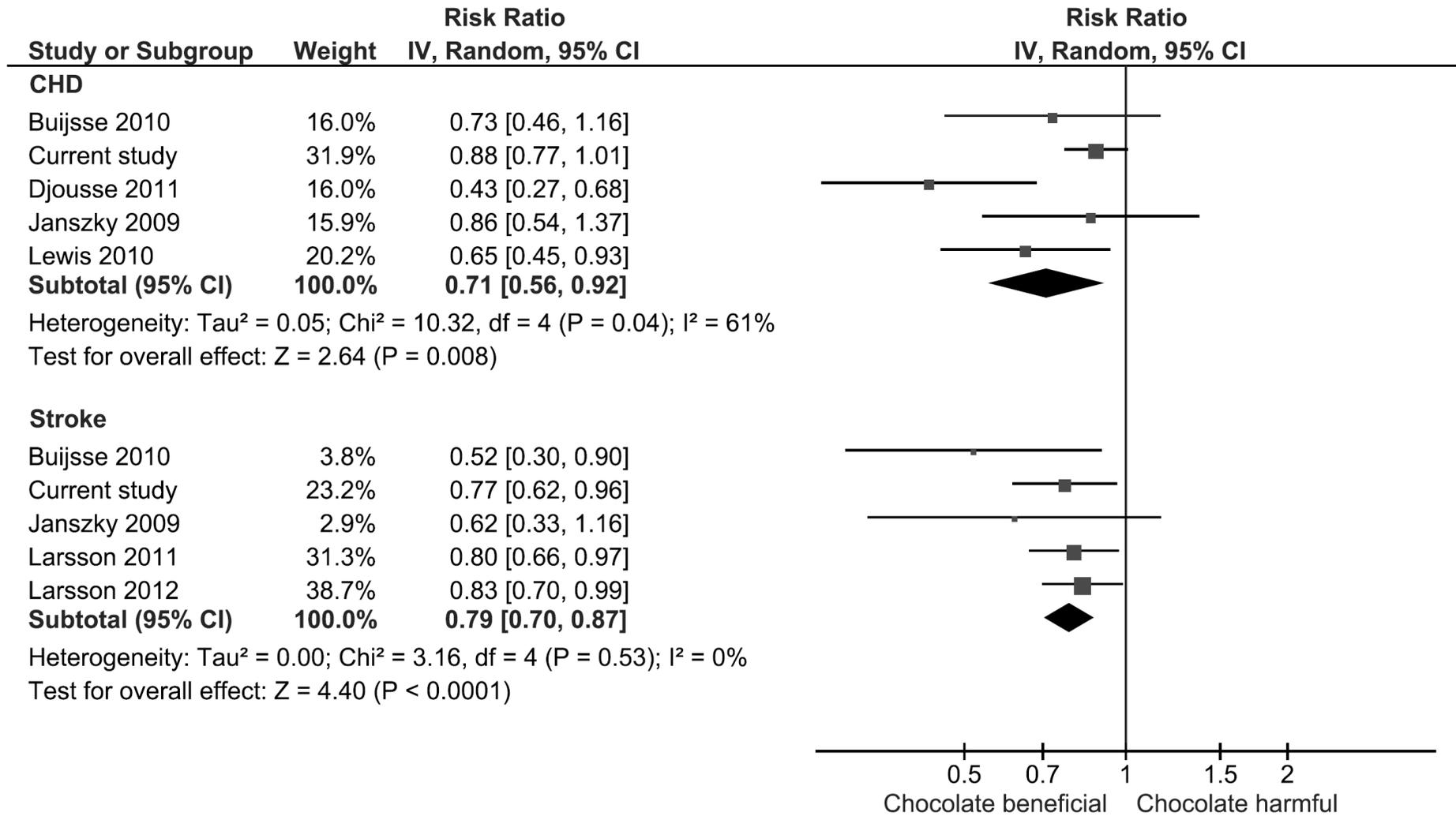| Study ID	Dietary exposures	Study design	No in analysis	Characteristics	Follow-up (years)	Outcome measures	Measures of association
Buijsse 2006	Cocoa intake	Prospective Cohort, Netherlands	470	White men with mean age 72 years.	15	Cardiovascular mortality at 15 years	Relative risk: 0.50 (0.32-0.78) for >2.25g/day vs. <0.5 g/day. (top vs. bottom tertile)
Buijsse 2010	Chocolate consumption	Prospective Cohort, Potsdam, Germany	19357	Men and women with mean age of 49 years, 38% were men and no history of myocardial infarction and stroke and not using blood pressure drugs.	10	Incident Myocardial infarction, stroke and composite of both at mean of 8.1 years follow-up	Relative risk: myocardial infarction 0.73 (0.47-1.18), stroke 0.52 (0.30-0.89), composite 0.61 (0.44-0.87) for 4th quartile vs. 1st quartile energy-adjusted chocolate consumption.
Djousse 2011	Chocolate consumption	Cross-sectional, United States	4970	Men and women with mean age of 52 years (range 25-93 years), 45% were men and 5% were of African-American origin.	NA	Prevalent Coronary heart disease (myocardial infarction or coronary revascularization)	Odds ratio: 0.43 (0.27-0.68) for >5 times a week vs. no consumption. (based on some degree of post-hoc categorization of collected data)
Janszky 2009	Chocolate consumption	Prospective Cohort. Sweden	1169	Men (71%) and women who survived an acute myocardial infarction with mean age of 59 years.	8	Cardiovascular mortality, recurrent myocardial infarction, stroke and congestive heart failure	Hazard ratio: CV mortality 0.34 (0.17-0.70), recurrent MI 0.86 (0.54-1.37), stroke 0.62 (0.33-1.16) for <2X week vs. never. (based on some degree of post-hoc categorization of collected data)
Larsson 2011	Chocolate consumption	Prospective Cohort, Sweden	33372	Women with no history of stroke, age range 49-83 years.	10.4	Incident Stroke	Relative risk: 0.80 (0.66-0.99) >45 g/week vs. <8.9 g/week. (top vs. bottom quartile)
Larsson 2012	Chocolate consumption	Prospective Cohort, Sweden	37103	Men with mean age mean of 59 years and no history of stroke.	10.2	Incident Stroke	Relative risk: 0.83 (0.70-0.99) for >51.6 g/week vs. <12 g/week (top vs. bottom quartile)
Lewis 2010	Chocolate consumption	Post-hoc analysis of a randomized trial of calcium supplementation in women, Australia	1216	Women, mean age 75 years	9.5	Atherosclerotic vascular disease and ischemic heart disease	Hazard ratio: Ischemic heart disease 0.65 (0.46-0.94) for <1 serving/week vs. >1 serving/week (based on some

Study ID	Dietary exposures	Study design	No in analysis	Characteristics	Follow-up (years)	Outcome measures	Measures of association
							degree of post-hoc categorization of collected data)
Mink 2007	Chocolate consumption	Prospective Cohort, USA.	34489	Women with mean age 62 years with no history of heart disease.	16	Coronary heart disease mortality and stroke mortality	Rate ratio: CHD mortality 0.98 (0.88-1.10), stroke mortality 0.85 (0.70-1.03) for <1 per week vs. > 1 per week (based on some degree of post-hoc categorization of collected data)
Current study	Chocolate consumption	Cohort	25663	55% women. 99.6% white Caucasians), mean age 59 years with no history of heart disease or stroke.	11.8	Incident coronary heart disease, stroke and composite of the two, defined as cardiovascular disease.	Hazard ratio: CHD 0.88 (0.77-1.01), stroke 0.77 (0.62-0.96), CVD 0.86 (0.76-0.97).(top quintile vs. bottom quintile)

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28   Figure 1: Meta-analysis risk of the association between chocolate consumption and risk of coronary heart disease and stroke.

Study or Subgroup	Weight	Risk Ratio IV, Random, 95% CI	Risk Ratio IV, Random, 95% CI
CHD			
Buijsse 2010	16.0%	0.73 [0.46, 1.16]	
Current study	31.9%	0.88 [0.77, 1.01]	
Djousse 2011	16.0%	0.43 [0.27, 0.68]	
Janszky 2009	15.9%	0.86 [0.54, 1.37]	
Lewis 2010	20.2%	0.65 [0.45, 0.93]	
Subtotal (95% CI)	**100.0%**	**0.71 [0.56, 0.92]**	

Heterogeneity: Tau² = 0.05; Chi² = 10.32, df = 4 (P = 0.04); I² = 61%
Test for overall effect: Z = 2.64 (P = 0.008)

Study or Subgroup	Weight	Risk Ratio IV, Random, 95% CI	Risk Ratio IV, Random, 95% CI
Stroke			
Buijsse 2010	3.8%	0.52 [0.30, 0.90]	
Current study	23.2%	0.77 [0.62, 0.96]	
Janszky 2009	2.9%	0.62 [0.33, 1.16]	
Larsson 2011	31.3%	0.80 [0.66, 0.97]	
Larsson 2012	38.7%	0.83 [0.70, 0.99]	
Subtotal (95% CI)	**100.0%**	**0.79 [0.70, 0.87]**	

Heterogeneity: Tau² = 0.00; Chi² = 3.16, df = 4 (P = 0.53); I² = 0%
Test for overall effect: Z = 4.40 (P < 0.0001)

0.5   0.7   1   1.5   2
Chocolate beneficial   Chocolate harmful

29

30

31   Figure 2: Meta-analysis of chocolate consumption and risk of cardiovascular disease (composite).

Study or Subgroup	Weight	Risk Ratio IV, Random, 95% CI
Any CV event		
Buijsse 2010	38.4%	0.61 [0.43, 0.86]
Current study	61.6%	0.86 [0.76, 0.97]
Subtotal (95% CI)	**100.0%**	**0.75 [0.54, 1.05]**

Heterogeneity: Tau² = 0.04; Chi² = 3.36, df = 1 (P = 0.07); I² = 70%

Test for overall effect: Z = 1.69 (P = 0.09)

CV mortality		
Buijsse 2006	32.5%	0.50 [0.32, 0.78]
Current study	46.2%	0.73 [0.60, 0.89]
Janszky 2009	21.3%	0.34 [0.17, 0.68]
Subtotal (95% CI)	**100.0%**	**0.55 [0.36, 0.83]**

Heterogeneity: Tau² = 0.09; Chi² = 6.01, df = 2 (P = 0.05); I² = 67%

Test for overall effect: Z = 2.81 (P = 0.005)

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33

34   Figure 3: Meta-analysis risk of coronary heart disease and stroke with chocolate consumption using propensity matched cohort.

Study or Subgroup	Weight	Risk Ratio IV, Random, 95% CI
CHD		
Buijsse 2010	16.6%	0.73 [0.46, 1.16]
Current study	29.9%	0.91 [0.77, 1.07]
Djousse 2011	16.6%	0.43 [0.27, 0.68]
Janszky 2009	16.4%	0.86 [0.54, 1.37]
Lewis 2010	20.6%	0.65 [0.45, 0.93]
Subtotal (95% CI)	**100.0%**	**0.72 [0.55, 0.93]**

Heterogeneity: Tau² = 0.05; Chi² = 10.80, df = 4 (P = 0.03); I² = 63%
Test for overall effect: Z = 2.48 (P = 0.01)

Study or Subgroup	Weight	Risk Ratio IV, Random, 95% CI
Stroke		
Buijsse 2010	4.2%	0.52 [0.30, 0.90]
Current study	16.6%	0.75 [0.57, 0.99]
Janszky 2009	3.2%	0.62 [0.33, 1.16]
Larsson 2011	34.0%	0.80 [0.66, 0.97]
Larsson 2012	42.0%	0.83 [0.70, 0.99]
Subtotal (95% CI)	**100.0%**	**0.78 [0.70, 0.88]**

Heterogeneity: Tau² = 0.00; Chi² = 3.24, df = 4 (P = 0.52); I² = 0%
Test for overall effect: Z = 4.27 (P < 0.0001)

35

Supplementary Figure 1: Love plot examining the standardized difference in covariates before and after propensity score adjustment.
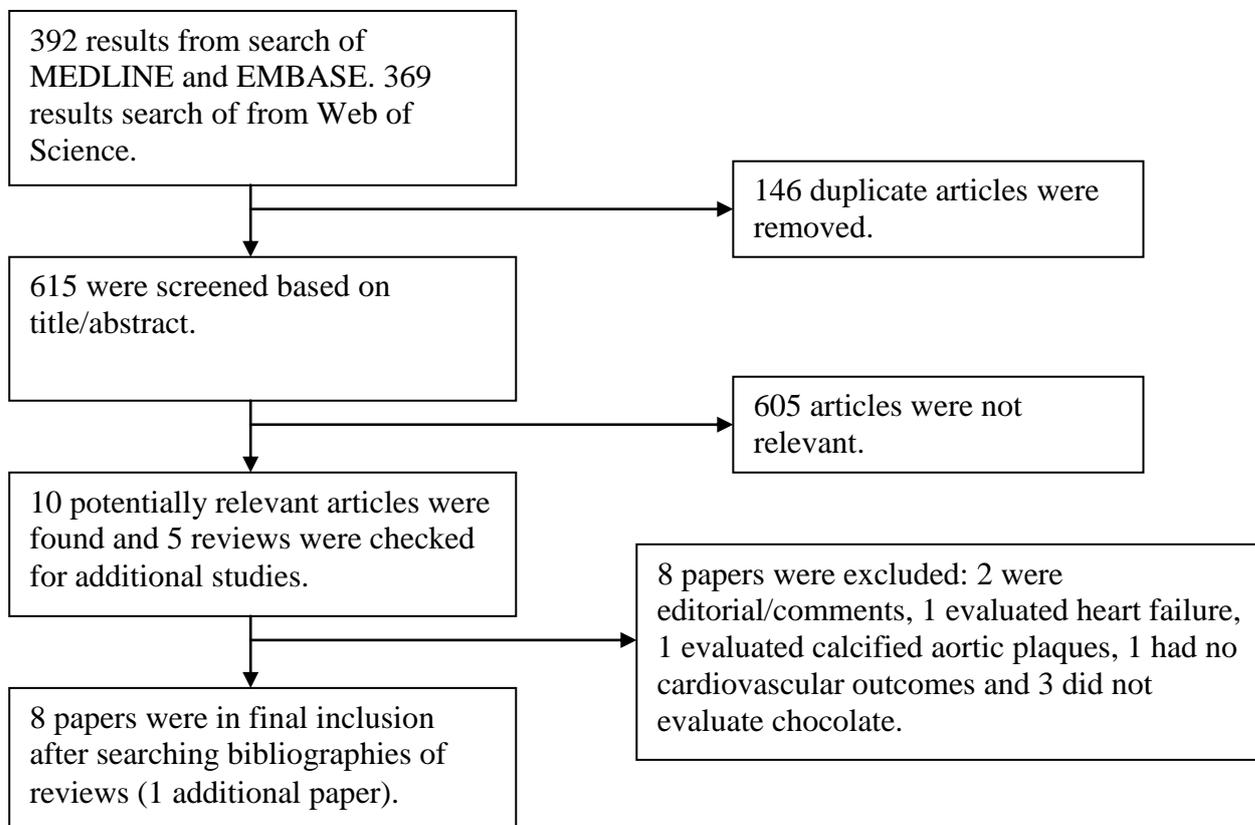
Supplementary Figure 2: Search results and study selection for meta-analysis of chocolate consumption and risk of cardiovascular disease.

392 results from search of MEDLINE and EMBASE. 369 results search of from Web of Science.

146 duplicate articles were removed.

615 were screened based on title/abstract.

605 articles were not relevant.

10 potentially relevant articles were found and 5 reviews were checked for additional studies.

8 papers were excluded: 2 were editorial/comments, 1 evaluated heart failure, 1 evaluated calcified aortic plaques, 1 had no cardiovascular outcomes and 3 did not evaluate chocolate.

8 papers were in final inclusion after searching bibliographies of reviews (1 additional paper).

Supplementary Table 1: **Baseline cardiovascular risk factors by quintiles of chocolate intake in 20,951 men and women of EPIC-Norfolk with 8,373 matched and unmatched on the propensity score (quintile 1 vs quintile 5), mean (sd) or percentage**

	Unmatched			Matched		
	Quintile 1	Quintile 5	p-value	Quintile 1	Quintile 5	p-value
Age	60.71(9.00)	57.07(9.39)	<0.001	58.64(8.91)	58.98(9.40)	0.19
Body mass index	26.34(4.05)	25.96(3.70)	<0.001	26.00(4.03)	26.00(3.63)	0.95
C-reactive protein	3.01(5.84)	2.91(7.45)	0.55	2.85(4.99)	3.03(8.10)	0.41
Diabetes mellitus (%)	7.31%	0.26%	<0.001	1.14%	1.25%	<0.001
Alcohol intake by HLQ	7.08(10.13)	6.99(9.14)	0.67	7.07(9.46)	6.91(9.00)	0.54
Carbohydrate intake by FFQ	231.78(76.06)	295.71(87.60)	<0.001	259.56(76.58)	257.01(64.72)	0.20
Protein intake by FFQ	78.88(20.89)	88.14(22.05)	<0.001	85.63(20.96)	79.74(18.14)	<0.001
Fat intake by FFQ	66.31(26.83)	91.43(31.29)	<0.001	76.36(27.32)	77.51(23.76)	0.11
HDL-cholesterol	1.43(0.43)	1.40(0.41)	<0.001	1.44(0.44)	1.42(0.41)	0.11
LDL-cholesterol	3.97(1.04)	3.94(1.02)	0.29	3.89(1.01)	4.00(1.03)	<0.001
Physical activity - Moderately inactive	26.76%	27.51%	0.53	28.41%	28.84%	0.80
Physical activity - Moderately active	20.60%	24.48%	0.006	23.30%	24.06%	0.79
Physical activity - Active	16.91%	21.65%	0.006	19.39%	18.74%	1.00
Sex (% men)	44.9%	52.1%	0.001	43.2%	42.5%	0.98
Smoking status (% current)	9.47%	14.1%	<0.001	11.8%	11.6%	0.93
Systolic blood pressure	136.75(18.61)	133.66(17.14)	<0.001	135.13(17.79)	134.61(17.65)	0.29

[a] propensity score based on the covariates used in Model 2: age, sex, smoking status, physical activity, FFQ energy and HLQ alcohol

[b] for comparison of characteristics before and after matching, derived by t-test

Supplementary Table 2: Hazard ratios of coronary heart disease, stroke and cerebrovascular disease (all and fatal) by quintiles of cocoa intake in 20,951 men and women of EPIC-Norfolk using propensity score analyses

	Quintile 1 vs 5	
	Events/N	Hazard ratio (95% CI)
CHD (fatal and non-fatal)		
Model 1	577/3618 vs 407/3771	0.85 (0.75-0.97)
Model 2	577/3618 vs 407/3771	0.88 (0.77-1.00)
Model 3	577/3618 vs 407/3771	0.91 (0.80-1.04)
Model 4a	435/2816 vs 280/2903	0.83 (0.71-0.97)
Model 4b	435/2816 vs 280/2903	0.82 (0.70-0.97)
Propensity score adjusted	435/2816 vs 280/2903	0.88 (0.76-1.01)
Matched by propensity score	307/2273 vs 283/2297	0.91 (0.77-1.07)
Stroke (fatal and non-fatal)		
Model 1	226/3969 vs 131/4047	0.78 (0.63-0.96)
Model 2	226/3969 vs 131/4047	0.78 (0.62-0.98)
Model 3	226/3969 vs 131/4047	0.80 (0.64-1.00)
Model 4a	168/3083 vs 99/3084	0.81 (0.63-1.05)
Model 4b	168/3083 vs 99/3084	0.81 (0.63-1.06)
Propensity score adjusted	168/3083 vs 99/3084	0.78 (0.62-0.99)
Matched by propensity score	125/2455 vs 94/2486	0.75 (0.57-0.99)
CVD (fatal and non-fatal)		
Model 1	729/3466 vs 500/3678	0.84(0.75-0.94)
Model 2	729/3466 vs 500/3678	0.86(0.76-0.97)
Model 3	729/3466 vs 500/3678	0.89(0.79-1.00)
Model 4a	548/2703 vs 350/2833	0.82(0.72-0.95)
Model 4b	548/2703 vs 350/2833	0.82(0.71-0.95)
Propensity score adjusted	548/2703 vs 350/2833	0.86(0.75-0.97)
Matched by propensity score	397/2183 vs 348/2232	0.87(0.75-1.00)
CVD (fatal)		

Model 1	308/3887 vs 162/4016	0.73(0.60-0.89)
Model 2	308/3887 vs 162/4016	0.76(0.62-0.92)
Model 3	308/3887 vs 162/4016	0.79(0.65-0.97)
Model 4a	231/3020 vs 113/3070	0.73(0.57-0.92)
Model 4b	231/3020 vs 113/3070	0.72(0.57-0.91)
Propensity score adjusted	231/3020 vs 113/3070	0.81(0.65-1.00)
Matched by propensity score	160/2420 vs 118/2462	0.77(0.60-0.97)

Model 1 adjusted for sex and age.

Model 2 adjusted for sex, age, smoking, physical activity, energy intake and alcohol consumption.

Model 3: as model 2 and diabetes, body mass index, systolic blood pressure, LDL-cholesterol and HDL-cholesterol.

Model 4a: as model 3, but restricted to number of participants for whom C-reactive protein is available.

Model 4b: as model 3 and C-reactive protein.

[a] Propensity scores are based on the covariates included in Model 2

Supplementary Table 3: Quality assessment of studies which evaluated chocolate consumption and cardiovascular disease.

Study ID	Ascertaining Chocolate Consumption	Determining Cardiovascular Outcome	Adjustment for Confounders
Buijsse 2006	The habitual dietary intake of the subjects was determined by interview conducted by experienced dieticians (using a cross-check dietary history method adapted to the Dutch situation). A total of 24 cocoa-containing foods and the intake of cocoa from individual foods was summed to yield actual cocoa in grams per day for each subject.	Information on the cause of death was obtained from hospital discharge data, general practitioners, and Statistics Netherlands. The final causes of death were ascertained by one clinical epidemiologist and coded according to the ICD-9.	Age; body mass index; smoking status; alcohol consumption; physical activity; aspirin use; anticoagulant use; diet prescription (Y/N); consumption of: vegetables, fruit, low and medium fat dairy, meat, sugar confectionery other than chocolate, cookies, savoury foods, nuts, and coffee; and total calorie intake
Buijsse 2010	Usual food intake in the year before baseline was assessed by a self administered 148-item food-frequency questionnaire. Chocolate consumption was asked by how frequent a chocolate bar of 50 g was consumed. Participants could indicate whether they consumed half, one, two, or three bars of chocolate.	Possible cases of MI or stroke were identified by self-reports or death certificates. Self-reported information was obtained by at least one of the four follow-up questionnaires, which contained questions about physician-diagnosed CVD and the use of medication. All possible cases were verified by reviewing medical records from the hospital, by contacting the patients' physician, or by review of the death certificate.	Age, sex, alcohol intake, employment status, body mass index, waist circumference, smoking status, occupational physical activity, sports cycling, education, and total energy intake
Djousse 2011	Dietary information collected through a staff-administered semi-quantitative food frequency questionnaire. Each subject was asked the following question: "In the past year, how often on average did you consume chocolate bars or pieces, such as Hershey's Plain, M & M, Snickers, Reeses; 1 ounce?". Possible answers were: ">6 per day, 4-6 per day, 2-3 per day, 1 per day, 5-6 per week, 2-4 per week, 1 per week, 1-3 per month, and almost never".	Prevalent CHD was assessed from the medical history and a 12-lead electrocardiogram. Individuals were defined as a case of CHD if there was a self-reported history of myocardial infarction, percutaneous transluminal coronary angioplasty, or coronary artery bypass graft that could be validated by review of medical records, or if abnormal Q waves detected on a resting 12-lead electrocardiogram.	Age, sex, dietary linoleic acid intake, education, exercise, smoking status, alcohol intake, fruit and vegetables intake, energy intake, non-chocolate candy intake
Janszky 2009	Questionnaire distributed few days after AMI regarding the number of usual (50 g) portions of chocolate that participants usually consumed per day, per week or per month during the last 12 months. The original consumption categories included: never, less than once per month, 1–3 times per month, once per week, twice per week, 3–4 times per week, 5–6 times per week, once per day, twice per day and 3 times or more per day.	All-cause and cardiac mortality (ICD-9 and -10) were used as primary end-points as provided by the National Cause of-death Register. Patients were also followed for nonfatal AMI using the Swedish Myocardial Infarction Register. Information on hospitalization for stroke was derived from the Swedish Hospital Discharge Register.	Age, sex, smoking status, obesity, physical inactivity, alcohol consumption (g/day), filtered coffee intake (cups/day), educational attainment, sweet score
Larsson 2011	Validated self-administered food-frequency questionnaire regarding how often on average they had consumed chocolate and 95 other foods during the previous year. There were 8 pre-defined consumption categories ranging from never to ≥3 times a day.	Incident cases of first stroke were ascertained by linkage with the Swedish Hospital Discharge Registry. The stroke events were classified by ICD-10 code. Information on dates of death was obtained from the Swedish Cause of Death Registry.	Age, education (less than high school, high school, or university), smoking status and pack-years of smoking (never; past <20, 20 to 39, or ≥40 pack-years; or current <20, 20 to 39, or ≥40 pack-years), body mass index (<20, 20 to 24.9, 25 to 29.9, or ≥30 kg/m2), total physical activity (metabolic equivalent of energy expenditure hours/day, quartiles), aspirin use (never, 1 to 6, ≥7 tablets/week), self-reported history of hypertension (yes or no), diagnosis of atrial

			fibrillation (yes or no), family history of myocardial infarction before 60 years of age (yes or no), and intakes of total energy (kcal/day), alcohol (non-drinkers or <3.4, 3.4 to 9.9, or ≥10.0 g/day), coffee (<1, 1 to 2, 3 to 4, or ≥5 cups/day), tea (0, <1, 1 to 1.9, 2 to 3.9, ≥4 cups/day), and quartiles (g/day) of fresh red meat, processed red meat, fish, fruits, and vegetables.
Larsson 2012	Validated self-administered food-frequency questionnaire that included 96 foods and beverages. Participants reported how often on average they had consumed chocolate based on 8 pre-specified consumption categories, ranging from never to 3 or more times per day.	Incident cases of first stroke were identified by linkage of the study population to the Swedish Hospital Discharge Registry and ICD-10 codes were used to identify stroke events in the cohort. Information on dates of death was obtained from the Swedish Death Register.	Age, education, smoking status, and pack-years of smoking, body mass index, total physical activity, aspirin use, history of hypertension, atrial fibrillation, family history of myocardial infarction, and intakes of total energy, alcohol, coffee, tea, fresh red meat, processed meat, fish, fruits, and vegetables.
Lewis 2010	At baseline, information on food and beverage consumption frequency was verified by their general practitioner and previously validated questionnaires. The frequency of chocolate consumption was collapsed into the following 3 categories: less than 1 serving/wk (rarely), 1 to 6 servings/wk (weekly), and 7 or more servings/wk (daily).	The Western Australian Data Linkage System (WADLS) was used to assess clinical outcomes. Atherosclerotic vascular disease events were defined using diagnosis codes from the ICD-10-AM.	Age, body mass index, socioeconomic status, and energy intake at baseline.
Mink 2007	Dietary assessment was performed using a 127-item food-frequency questionnaire was adapted from the questionnaire used in the 1984 Nurses' Health Study survey which had detailed information on fruit (15 items) and vegetables (29 items) and included information on individual foods with high flavonoid content (eg, chocolate).	Women were followed annually through the State Health Registry of Iowa and identified through the 4 follow-up questionnaires by linking women who did not respond to the questionnaire with the National Death Index. The ICD-9 was used to classify the reported cause of death.	Age, energy consumption, marital status, education, blood pressure, diabetes, body mass index, waist to hip ratio, physical activity, smoking, oestrogen use
Current study	Three questions from a food frequency questionnaire were considered indicative of chocolate consumption namely "Chocolates singles or squares" (average portion size of 8 g), "Chocolate snack bars, e.g. Mars, Crunchie" (average portion size of 50 g) and "Cocoa, hot chocolate (cup)" (average portion size of 12 g powder weight). Frequency categories were multiplied by the portion size to derive the amount of chocolate products eaten (g/day). The sum of the weights of these food items consumed, rather than their flavonoid or cocoa content, formed the measure of exposure.	Patients admitted to hospital were identified using their National Health Service number by data linkage with ENCORE. All participants were flagged for death certification at the UK Office of National Statistics, ascertaining vital status for the entire cohort. Diagnosis of CHD, stroke and CVD (combination of CHD and stroke) was based on ICD-10 codes.	Age, sex, smoking status, body mass index, systolic blood pressure, LDL-cholesterol, HDL-cholesterol, C-reactive protein, physical activity, protein intake, total fat intake, total carbohydrate intake and alcohol intake.

Supplementary Table 4: Use of adjustments or exclusions in studies which evaluated chocolate consumption and cardiovascular disease.

Study ID	Diabetes mellitus	Body Mass Index	Total Energy Intake	Smoking	Blood Pressure	Cholesterol
Buijsse 2006	Y (excluded patients with diabetes)	Y	Y	Y	N	N
Buijsse 2010	Y	Y	Y	Y	N	N
Djousse 2011	Y	N	Y	Y	N	N
Janszky 2009	Y (excluded patients with diabetes)	N	N	Y	N	N
Larsson 2011	Y (excluded patients with diabetes)	Y	Y	Y	N	N
Larsson 2012	Y (excluded patients with diabetes)	Y	Y	Y	Y (hypertension)	N
Lewis 2010	N	Y	Y	N	N	N
Mink 2007	Y	Y	Y	Y	Y	N
Current study	Y	Y	Y	Y	Y (systolic blood pressure)*	Y*

*Although these factors were included in the study, the model without these risk factors were included in the meta-analysis.

Data Supplement 1: Search strategy for chocolate consumption and risk of cardiovascular disease

EMBASE,MEDLINE; Duplicate filtered: [((cacao OR chocolate OR cocoa) AND

((cardiovascular disease) OR (coronary heart disease) OR (ischaemic heart disease) OR

(ischemic heart disease) OR stroke OR (brain vascular accident) OR (cerebrovascular

accident) OR (cerebral vascular accident))).ti,ab]; 392 results.