# Effect of intake of apples, pears or their products on cardiometabolic risk factors and clinical outcomes: a systematic review and meta-analysis

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Abbreviations: CVD, cardiovascular disease; CMD, cardiometabolic disease; T2DM, type 2 diabetes mellitus; RCT, randomized controlled trial; QALY, quality adjusted life year; BMI, body mass index; TC, total cholesterol; RR, relative risk

Short running head: Apple intake and cardiovascular disease

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## ABSTRACT

Apples and pears contain nutrients that have been linked to cardiovascular health. We conducted a systematic review and meta-analysis to summarize related research. Medline, Cochrane Central, and Commonwealth Agricultural Bureau databases were searched for publications on apple or pear intake and CVD/CMD. Studies in adults (healthy or at risk for CVD) that quantified apple or pear intake were included. Random-effects models meta-analysis was used when ≥3 studies reported the same outcome. 22 studies were eligible including 7 RCTs, 1 non-randomized trial, and 14 prospective observational studies. In RCTs, apple intake significantly decreased BMI, but made no difference in body weight, serum lipids, blood glucose, or blood pressure. In observational studies, apple or pear intake significantly decreased risk of cerebrovascular disease, cardiovascular death, T2DM, and all-cause mortality. No association was reported for cerebral infarction or intracerebral hemorrhage. In conclusion, apple or pear intake significantly decreased BMI and risk for CVD outcomes.

Keywords: apples, pears, cardiovascular disease, cerebrovascular disease, BMI, type-2 diabetes mellitus

## INTRODUCTION

Fruit intake is associated with a decreased risk of cardiovascular disease (CVD) events and risk factors in several epidemiological studies (1-5). The Dietary Guidelines for Americans 2015-2020 recommends a high intake of fruit, as part of a healthy eating pattern for the prevention of chronic disease (6). Few Americans eat adequate servings of fruit to adhere to this recommendation, however, apples are the most consumed and

the fourth least expensive type of fresh fruit in the US (7-9). A better understanding of the health effects of apple intake as well as intake of fruits with similar nutrient content, such as pears, aside from total fruit, on CVD could be useful in informing federal nutrition guidance around fruit, considering apples' popularity and accessibility.

CVD risk factors and events are highly prevalent in the US, and the health and financial burden of this disease warrants the investigation of prevention through diet (10). Each year, nearly one in every six US healthcare dollars is spent on treatment for CVD (11). Direct healthcare costs attributable to CVD were $193 billion and the associated cost due to productivity loss were $123.5 billion in 2012 (11). The health benefits associated with fruit consumption could result in considerable cost savings, through possible reduction of medications, invasive interventions, and lost productivity.

Apples and pears contain several bioactive compounds that have been individually associated with a decreased risk for CVD risk factors and events including flavonoids, dietary fiber and antioxidants (12-15). Intake of dietary fiber and antioxidants from fruits has been found to be significantly associated with a decreased risk for CVD (14-17). In an analysis of the CPS II Nutrition Cohort, men and women with the highest intake of flavonoids had an 18% risk reduction for CVD mortality (12).

While the cardiovascular health benefits of the bioactive compounds found in apples and pears are widely recognized, the effect of intake of whole apple, pear, and their products has remained somewhat inconclusive. Several studies have found a significant CVD risk reductions due to apple or pear intake (16, 18-21) , but not all studies (22). A recent systematic review and meta-analysis of observational studies indicated a protective relationship between apple and pear intake and risk of T2DM, a highly prevalent and costly risk factor for CVD (23). To the best of our knowledge, a systematic review and meta-analysis on apple and pear intake and CVD has not been previously conducted, and this study aims to fill this gap in the literature.

## METHODS

We conducted a systematic review of published literature evaluating the effects of apple intake on CVD and CMD risk factors and events. We developed a single causal pathway, or analytical framework, depicting the potential association between intake of apple, pear and their products and CVD/CMD risk factors and outcomes to guide our review (**Supplemental Figure 1**). The systematic review results were reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Statement (24).

### Data Sources and Study Eligibility

We conducted a comprehensive literature search in MEDLINE, Cochrane Central, and Commonwealth Agricultural Bureau abstracts from 1946 through August 2019 for publications that measured apple or pear intake and CVD/CMD clinical outcomes and risk factors in adults (**Supplemental Table 1**). No language restriction was applied during searches. Citations were screened in duplicate using the predefined study eligibility criteria and discrepancies were resolved by consensus in group conferences.

#### *Study inclusion criteria*

We included prospective cohorts and intervention trials conducted in adults ≥ 18 years of age that quantified the amount of apple or pear intake. We included studies that examined pear or combined apple and pear intake because of similarities in nutrient content between apples and pears and because apple and pear intake is often combined in analysis of nutrition data (51). Studies were eligible if the population was either healthy or had CVD/CMD risk factors (i.e., hypertension, hyperlipidemia, metabolic syndrome, or diabetes) at baseline. Studies with no apple or pear intake or low intake of apples or pears as comparators were accepted. The clinical outcomes of interest included any CVD, acute coronary syndrome, myocardial infarction, ischemic heart disease, stroke, coronary artery disease, atrial fibrillation, all-cause mortality, cardiovascular

mortality, cerebrovascular disease, cerebral infarction, and heart failure. CMD risk factors of interest included metabolic syndrome, type 2 diabetes, blood glucose, hypertension, systolic blood pressure (SBP), diastolic blood pressure (DBP), hypercholesterolemia, blood lipids (total cholesterol [TC], high density lipoprotein [HDL-C], low density lipoprotein [LDL-C], very-low density lipoproteins [VLDL-C], triglycerides, LDL-C/HDL-C, TC/HDL-C), body weight, body mass index (BMI), and waist-hip ratio.

***Study exclusion criteria***

We excluded studies that evaluated apple pectin, or apple pomace. We also excluded studies in children and pregnant women. The following study designs were excluded: retrospective, cross-sectional, case-reports, and single-arm (interventions with no control group), mixed intervention, pharmacokinetic, in-vitro, and cell-culture studies. In addition to the above common eligibility criteria, we established additional criteria specific to study design.

***Eligibility Criteria for Intervention Trials***

In our analysis of intervention trials, we included studies with known doses of apples, pears, and their products. The minimum intervention duration was at least one week for recorded blood glucose levels and at least three weeks for other risk factor outcomes. Studies with <5 subjects per arm were excluded.

### *Eligibility Criteria for Cohort Studies*

In our analysis of cohort studies, we included studies with reported intake of apple, apple products, pear, pear products, or combined apple and pear. Studies that reported multivariable results adjusting for any potential confounders were eligible. Studies with at least 6 months of follow-up time were included.

### Data Extraction and Quality Assessment

Data from each study was extracted independently by one of six investigators and reviewed and confirmed by at least one other team member. Any conflicts regarding extraction were resolved in team discussions. The extracted data table included: study design, intervention description, population characteristics, methods for controlling for potential confounders or effect modifiers, outcomes, and results depicting associations between apple or pear intake and specific outcome of interest.

We assessed the methodologic quality of each study based on predefined criteria, in accordance with the Agency for Healthcare Research and Quality's suggested methods for systematic reviews (25). Study quality was determined in duplicate and discrepancies were resolved by consensus in group discussion. We applied risk of bias items in the modified Newcastle-Ottawa Scale (26) for observational studies, the Cochrane risk of bias for clinical trials (27), and nutrition–specific items for a critical appraisal of micronutrient systematic reviews for both clinical trials and observational studies (28).

### Data Synthesis

Analyses were conducted separately for intervention trials and observational studies. We performed random-effects model meta-analyses when similar data from ≥ 3 studies were available (29). For intervention trials, we combined net differences (Net change = (apple $\text{intake}_{\text{final}}$ – apple

$intake_{initial}$) – ($control_{final}$– $control_{initial}$)) for continuous outcomes. We tested between-study heterogeneity with the Q statistic (significant when $p < 0.10$) and quantified its extent with $I^2$. An $I^2$ value of 25%, 50%, and 75% was respectively considered low, moderate, or high heterogeneity (30).

For observational studies, we synthesized relative risks (RRs) comparing the extreme categories of apple or pear intake (highest compared with lowest, as defined within each study) provided that the categories corresponded to similar doses of intake across studies. We performed sensitivity analyses when there were studies reporting various similar doses of apple or pear intake (including a comparison of medium intake to low intake) or if they reported data for subgroups, such as sex. All statistical analyses were conducted in Stata version 14 (StataCorp). Forest plots were created using R version 3.3 (R Core Team).

For studies that reported quantities by serving size, we considered a serving equivalent to one medium-sized apple or pear. For a study that reported intake of apple intake as catechin content, we used the USDA Food Composition Database (51) to obtain the total catechin composition of a medium-sized apple and back calculated the amount of apple consumed for each quartile.

All included studies that could not be combined quantitatively in meta-analyses were summarized in narrative form and in tables that tabulated the important features of the study populations, design, intervention, outcomes, and results. Summary tables were organized by outcomes of interest.

## RESULTS

Database searches identified 1834 citations. Full-text articles of 66 citations that were accepted in abstract screening were retrieved and reviewed against eligibility criteria. Full-text screening yielded 28 eligible articles (**Figure 1)**. Of 28 eligible articles, 11 published intervention trial data and 17 published prospective cohort data. No foreign language publications met the inclusion criteria.

**Intervention trials**

Eight intervention trials (7 RCTs and 1 non-randomized trial) in 11 publications that enrolled 596 participants were eligible. Trials were conducted in Brazil (n=1), Denmark (n=1), Germany (n=1), Iran (n=1), Ireland (n=1), Norway (n=1) and the USA (n=1), and the location was not reported in one trial. The duration of the trials ranged from 4 to 20 weeks. Two trials included only men, three included only women, and three trials recruited both men and women. Of seven RCTs, five were parallel-arm randomized trials and two were crossover trials. Included participants in RCTs varied considerably; they were either healthy (31, 32), non-diabetic (33) or had increased risk of CVD/CMD (19, 32, 34, 35). The apple or pear interventions and their comparators varied across RCTs. Apple or pear intake ranged from 75 to 900 grams/day and comparators included no apple or pear, control beverages with equivalent amounts of calories and fructose, dried plums, oat cookies, and kiwis. In addition to 12 week time points, one RCT reported long-term data at 6 and 12 months. Meta-analyses of RCTs were conducted for the following outcomes: body weight (5 RCTs (31-34, 36)), BMI (3 RCTs (31-33)**),** HDL-C (4 RCTs (19, 31, 33, 36), TC (5 RCTs [19, 32-35]), LDL-C (4 RCTs (19, 31, 33, 36)), and TG 5 RCTs ((19, 31, 33, 36)). No meta-analysis was conducted for SBP, DBP, waist circumference, waist to hip ratio, LDL-C:HDL-C, TC:HDL-C, glucose and glucose:insulin ratio as these outcomes were reported in less than three RCTs. Baseline details for intervention trials can be found in **Table 1**, the meta-analysis results are tabulated in **Table 2,** the risk of bias of each study is listed in **Supplemental Table 2**, and meta-analysis results of trials reporting the effect of apple in % net change is tabulated in **Supplemental Table 3**.

## Body Weight-related Outcomes

### Body Mass Index

Three RCTs with a total of 229 participants (31-33) reported the effect of apple intake on BMI. Meta-analysis of the three trials comparing apple with a variety of controls found a significant decrease in BMI (summary net change: -0.39; 95% CI: -0.59, -0.20) without heterogeneity ($I^2$ 0.0%; p = 0.934) (**Figure 2**).

### Body weight

Five trials included a total of 393 participants (31-34, 36) reported the effect of apple intake on body weight. Meta-analysis of the five trials, which compared apple with different controls, found no difference in body weight (summary net change: 0.14; 95% CI:-0.45, 0.73; $I^2$ = 0.0%; p = 1.00) between groups. Sensitivity analysis using long-term (6 month and 12 month results) data found similar results (31).

### Waist-to-hip ratio

One crossover RCT conducted in Denmark among 23 healthy men and women (36) measured the effect of apple intake with waist-to-hip ratio and found no difference between groups. A separate crossover RCT reported the effect of pear intake on waist-to-hip ratio (35) conducted among 43 adults at risk for CVD. Waist-to-hip ratio among the group consuming pears was significantly reduced compared to the control group at both 6 and 12 week time points.

## Serum Lipid Outcomes

### Total Cholesterol

Five RCTs (19, 31-33, 36) and one nonrandomized trial (37) investigated the effect of apple intervention on serum TC. Meta-analysis of five RCTs with a total of 324 subjects found no effect on TC (summary net change: -4.10; 95% CI: -13.02, 4.82) with a moderate heterogeneity ($I^2$ = 46.0%, p = 0.116) (**Figure 3**). Sensitivity analysis using the additional time points of 6 months (net change:-5.14; 95% CI: -16.31, 6.03; $I^2$= 64.7%) and the 12 months (net change: -4.50; 95% CI: -14.45, 5.45; $I^2$ = 54.8%) also found no effect of apple on serum TC.

In the single nonrandomized trial of 70 men that used a time point of 16 weeks, there was a significantly lower TC in the apple intake group versus the control group (37).

### High Density Lipoprotein Cholesterol

Four RCTs (19, 31, 33, 36) and one nonrandomized trial (37) reported on the effect of apple intake on serum HDL-C levels. Meta-analysis of the four RCTs with a total of 289 participants found no difference in HDL-C between apple intake and control groups (net change: -0.79; 95% CI: -2.58, 0.99; $I^2$ = 0.0%, p = 0.726). Sensitivity analyses using 6 month (net change: -0.97; 95% CI: -2.76, 0.82; $I^2$ = 0.0%, p = 0.817) and 12 month time points (net change: -0.75; 95% CI: -2.55, 1.05; $I^2$ = 0.0%, p = 0.647) also found no difference in HDL-C between apple intake and control (31).

In the single nonrandomized trial of 70 men that compared two apples per day versus three apples per week for 16 weeks found a significant increase in HDL-C with apple intake versus control (38).

**Low Density Lipoprotein Cholesterol**

Four trials (19, 31, 33, 36) that included a total of 175 participants reported on the effect of apple intake on LDL-C. The main meta-analysis found no difference in LDL-C (summary net change: −4.75 mg/dL; 95% CI: −10.40, 0.90; $I^2$ = 19.0%) between apple intake and control. In sensitivity meta-analyses using 6-month (summary net change: -7.09; 95% CI: -15.54, 1.35; $I^2$ = 61.4) and 12-month time points (summary net change: -5.29; 95% CI: -11.77, 1.18; $I^2$ = 34.4%) found no difference in LDL-C (31).

**Triglycerides**

Five RCTs (19, 31-33, 36) reported the association between apple intake and TG. A meta-analysis of RCTs with a total of 324 subjects compared apple intake with a heterogeneous control group and found no difference in TG (net change: 8.91 mg/dL; 95% CI: -10.32, 28.14; $I^2$ = 59.1%, p = 0.044). Sensitivity analysis using 6 month (net change: 9.66 mg/dL; 95% CI -9.05, 28.36; $I^2$ = 54.0%) and 12 month time points (net change 8.60 mg/dL; 95% CI -14.93, 32.13; $I^2$ = 72.4) found no difference in LDL-C between groups (31).

**Other Serum Lipid Outcomes**

One parallel RCT (19) reported the effect of apple intake for 8 weeks on VLDL-C in 46 hyperlipidemic, overweight men. This RCT found that there was no difference in VLDL-C between apple and control groups. Two RCTs (19, 31) that reported on the effect of apple intake on LDL-C:HDL-C found no significant difference between groups.

**TC:HDL-C**

Two RCTs (31, 36) that included a total of 206 healthy participants found no difference in TC:HDL-C between apple intake versus control.

**Apolipoprotein B**

One RCT (19) that included 46 hyperlipidemic men measured the effect of apple intake on apolipoprotein B. There was no significant difference in apolipoprotein B levels between the groups.

**Systolic and Diastolic Blood Pressure**

Two RCTs (34, 36) reported the effect of apple intake on SBP and DBP. Among 115 hypertensive participants, one RCT (34) found no difference in SBP (net change: -0.6; 95% CI -4.7,3.5; p = 0.825) or DBP (net change: 1.7; 95% CI -0.8, 4.2; p = 0.177) between groups.

Another RCT (36) that included 23 healthy men and women, found a statistically significant reduction for both SBP (net change: -3.93 mm Hg; 95% CI: -7.60, -0.26; p = 0.04) and DBP (net change: -2.96 mm Hg; 95% CI: -5.97, – 0.05; p = 0.05) after 4 weeks of apple intervention compared with control.

One crossover RCT (35) reported the effect of pear intake on SBP. Among 43 adults at risk for CVD, SBP tended to be reduced at 12 weeks while no changes were observed in the control group. A second publication reporting results for the same RCT (38)reported the effect of pear intake on SBP and DBP. Among 36 adults at risk for CVD, SBP was significantly lower than baseline levels among the pear group, while no changes were observed for the control group. No changes were observed in DBP for either group.

**Glucose Metabolism Outcomes**

Three RCTs (32, 36, 39) with a total of 236 participants reported the effect of apple intake on insulin levels and found no difference in insulin levels between groups. A meta-analysis was not possible because one trial only reported qualitative results (39). Two RCTs (32, 39) that reported the effect of apple intake on blood glucose levels and one RCT (32) that reported on glucose:insulin found no difference between groups.

**Cohort studies**

A total of 14 cohorts were evaluated in 17 publications and examined the following outcomes: acute coronary syndrome (1 cohort (20)), cerebrovascular disease (4 cohorts (17, 21, 40, 41)), diabetes (5 cohorts in 4 studies (42-45)), CVD mortality (3 cohorts (14, 18, 46, 47)), all-cause mortality (3 cohorts (18, 46, 48)), hypertension (3 cohorts in 1 study (49)), and body weight (3 cohorts in 1 study (50)). Studies were conducted in the USA [n=5], Denmark [n=1], Finland [n=1], Sweden [n=3], The Netherlands [n=2], Australia [n=1], and USA/China [n=1]. Three cohorts reported results for at least one outcome stratified by sex. Six cohorts included only women, two included only men, and seven included both men and women. Three publications reported combined results for Nurses' Health Study (NHS), Nurses' Health Study II (NHS II), and Health Professionals Follow-Up Study (HPFS) and stratified their results by cohort. Nine of 14 cohorts reported results for apple and pear intake and the remaining five cohorts reported only apple intake. Studies varied in the length of follow-up and in the amount of apple or pear intake (high, medium and low intake). Follow-up ranged from 4 to 28 years. High apple or pear intake ranged from to 35.2 to more than 100 grams/day, medium apple or pear intake ranged from 0 to 100 grams/day, and low apple or pear intake ranged from 0 to 35.2 grams/day. Baseline details for studies can be found in **Table 3**, the meta-analysis results are tabulated in **Table 4**, and risk of bias is listed in **Supplemental Table 4**.

**Acute coronary syndrome**

The Danish Diet, Cancer and Health Study cohort (20) followed 55,338 men and women between the ages of 50-64 for a median of 7.7 years and reported a significantly decreased risk of ACS (Incidence rate ratio (IRR): 0.78, 95% CI: 0.64, 0.95) in men with high apple intake. However, no association was observed in women (IRR: 0.93, 95% CI: 0.67, 1.29). Additional analyses found that for every 25 g/day increase in apple intake, men had a decreased risk of ACS (IRR: 0.97, 95% CI: 0.94, 0.99), but women did not (IRR: 0.97, 95% CI: 0.93, 1.01).

**Cerebrovascular disease**

Four studies (17, 21, 40, 41) reported an association between apple intake and cerebrovascular disease, including total and acute stroke. All studies included both men and women, and only one study (17) stratified results by men and women (reporting 2 data points).

Meta-analysis of three studies with a total of 139,507 participants found a significantly decreased risk of cerebrovascular disease (summary adjusted RR: 0.89; 95% CI 0.83, 0.95) without heterogeneity ($I^2$ = 0.0%; p = 0.58) (**Figure 4)**. A fourth study (40), conducted in The Netherlands, followed 20,069 participants for 10.3 years and found that each 25 g/day increase in intake of apple was inversely associated with stroke (HR: 0.93; 95% CI: 0.86-1.00).

Meta-analysis of two studies (with three data points) (17, 21) with a total of 84,169 participants found no difference in risk for cerebral thrombosis or embolism between highest versus lowest quantiles of apple intake (summary adjusted RR: 0.76; 95% CI: 0.55, 1.06; $I^2$ = 52.3%; p = 0.123).

Meta-analysis of three studies (with a total of 139,507 participants (17, 21, 41) found no difference in risk of intracerebral hemorrhage (summary adjusted RR: 0.92; 95% CI: 0.76, 1.12; $I^2$ = 0.0%) when comparing the highest versus lowest quantile of apple intake.

**Cardiovascular death**

Meta-analysis of four studies (14, 18, 46, 47) with a total of 36,753 participants found a significant decrease in cardiovascular death (summary adjusted RR: 0.86; 95% CI: 0.78, 0.95) without any heterogeneity ($I^2$ = 0.0%; p = 0.387).

**All-cause mortality**

Meta-analysis of three studies with a total of 55,625 participants (18, 46, 48) found a significant decrease in all-cause mortality (summary adjusted RR: 0.85; 95% CI: 0.77, 0.92) with low heterogeneity ($I^2$ = 0.0%; p = 0.49) comparing the highest intake to the lowest intake of apple (**Figure 5**). Death was ascertained using data from the Central Statistical Office of Finland (46), the Swedish Bureau of Statistics and Cause of Death Registry (48), or the Western Australian Mortality Database (18).

**Type 2 Diabetes**

Meta-analysis of the six cohorts in four publications (42-45) included 339,383 participants and found a significant decrease in risk of diabetes (summary adjusted RR: 0.86; 95% CI 0.77, 0.95) with a high heterogeneity ($I^2$ = 78.0%; p = 0.001) comparing the highest to the lowest quantile of apple or pear intake (**Figure 6**). In sensitivity analysis, four cohorts that included only women or that reported results stratified by sex (42-44) also found a significant decrease in risk of diabetes (summary adjusted RR: 0.81; 95% CI: 0.68, 0.96) with a high heterogeneity ($I^2$ = 89.7%; p = 0.001).

Additional sensitivity analyses comparing mid-quantiles (42-44) to the lowest quantile of apple intake found a significant decrease in the risk of diabetes (summary adjusted RR: 0.88; 95% CI 0.80, 0.96; $I^2$ = 84.2%; p = 0.001) overall and for a female-only subgroup (summary

adjusted RR: 0.86; 95% CI 0.74, 0.98; $I^2$ = 87.9%; p = 0.001). Meta-analysis comparing medium to low levels of apple intake also found a significant decrease in risk of diabetes (summary adjusted RR: 0.92; 95% CI 0.86, 0.99; $I^2$ = 74.9%; p = 0.001), but not a significant decrease among the female-only subgroup (summary adjusted RR: 0.92; 95% CI 0.82, 1.02; $I^2$ = 81.3%; p = 0.001).

**Body weight**

One study (50), comprised of three prospective cohorts (NHS, NHS II, and HPFS) with a total of 133,468 participants found an association between increased intake of apple and a decrease in body weight by an average of -1.24 lbs (95% CI: -1.62, -0.86) after 4 years. In each of the cohorts, following apple intake, body weight decreased an average of -1.43 lbs (95% CI: -1.62, -1.23) in NHS, -0.85 lbs (95% CI: -1.06, 0.64) in NHS II, and -1.45 lbs (95% CI: -1.65, -1.25) in HPFS participants.

**Hypertension**

In one study, the hazard ratios for incident hypertension comparing the highest and lowest quartiles of apple intake for the NHS, NHS II and HPFS cohorts were 0.90 (95% CI 0.85-0.96, p = 0.003), 0.93 (95% CI 0.87-0.99, p = 0.07) and 0.93 (95% CI: 0.86-1.00, p = 0.03), respectively (49). The pooled hazard ratio for incident hypertension, comparing the highest quartile to the lowest quartile was 0.91 (95% CI: 0.88-0.95, p < 0.001).

## DISCUSSION

This systematic review has demonstrated significant reduction in the risk of cardiovascular outcomes associated with apple intake in cohort studies and for the outcome of BMI in RCTs. To the best of our knowledge this is the first meta-analysis of the impact of apple intake on a comprehensive list of CVD/CMD risk factors and outcomes in cohort studies and in RCTs. Our review found that higher intake of apple, pear, or

combined apple and pear was associated with a significant decrease in BMI in RCTs, and a significant decrease in the risk of cerebrovascular disease, CVD/CMD mortality, diabetes, and all-cause mortality in observational studies. There was no difference between apple/pear and control groups for the outcomes of body weight, HDL-C, LDL-C, TC, and TG in meta-analysis of RCTs, or the risk of cerebral infarction and intracerebral hemorrhage in meta-analysis of cohort studies. While the meta-analysis included between three and five studies, the number of included participants varied considerably across RCTs and cohort studies. In RCTs, the included participants ranged between 175 and 393, and in cohort studies the participants ranged between 55,625 and 284,323. Further, individual studies did not find a significant change in waist-to-hip ratio (n = 1), apolipoprotein b (n = 1), TC:HDL-C (n = 2), glucose:insulin (n=1), insulin (n = 3), and VLDL-C (n = 1). Studies had conflicting results for LDL-C:HDL-C (n = 2), glucose (n=2), SBP (n=2), DBP (n=2), and ACS (n = 1).

The protective association observed in this study between apple intake and diabetes is consistent with observations made in a previous systematic review and meta-analysis (23). Though similar, our estimation of the effect of apple intake on diabetes was stronger than the previous review (RR of 0.77 compared to RR of 0.82). Unique to this study, we also conducted a sensitivity analysis on cohort studies that only included women and separate meta-analyses of RCTs (23). Our findings are consistent with another review that observed protective associations between increased fruit intake and heart disease outcomes (51). However, our review focused solely on apple, pear, or combined apple and pear exposure while others reviewed studies with a variety of fruit exposures. To the best of our knowledge, our findings of the effect of apple intake on other CVD/CMD risk factors were not addressed in previous systematic reviews and meta-analyses.

The significant association that we demonstrate in this review between apple and pear intake and cardiovascular outcomes in cohort studies is consistent with studies demonstrating the health benefits of apples, possibly due to the presence of phytochemicals like flavonoids, anthocyanins and other antioxidants (13). Apples and pears are a rich source of flavonoids, which have documented cardiovascular benefits (12).

The significant association observed between BMI reduction and apple intake in the RCTs is consistent with the results of the meta-analyses of cohort studies in that reduction in BMI correlates strongly with improved cardiovascular outcomes (52). However, we did not observe significant associations among other cardiovascular risk factors with apple intake including serum lipid levels.

In the results reported above, we combined apple and pear intake, because of similarities in nutrient content between apples and pears (53). We examined the validity of this approach in both the RCTs and the cohort studies. In the RCTs, only one study that was included in meta-analysis reported results for apple and pear as one of the arms (34). Repeating the meta-analyses for the apple-only subgroup showed a similar trend for the remaining 6 studies that were included in this meta-analysis (**Supplemental Table 6**). Nine out of fourteen cohort studies grouped apple and pear intake together into one exposure category. When we conducted a subgroup analysis of the five studies that studied only apple intake, meta-analyses could be performed only for Type 2 Diabetes incidence and all-cause mortality. For the other outcomes, there were not enough studies available for apple-only analyses. For the two outcomes mentioned, the results had similar trends (**Supplemental Table 5**) compared to the results obtained when including studies where apple and pear intake was combined.

In the meta-analyses reported for cohort studies above, we combined studies regardless of whether the studies controlled for diet or not. However, when we performed a sub-group analysis using only those studies that controlled for diet, the results showed a similar trend as seen when all studies were included (**Supplemental Table 7**).

The limitations of this review are largely reflective of the quality of primary studies as well as the consistency of data collection and analysis methods in individual studies. Many of the included observational studies used data collection and outcome assessment methods that are prone to biases, including self-report for dietary and outcome assessment as well as record linkage for outcome assessment (54). Secondly, the populations examined in some of the included cohort studies were restricted to nurses or other health professionals who may not be representative

of the average individual, potentially limiting the generalizability of the results. There is also a potential for a substitution effect in that subjects who consumed more apples may also have a diet that may substantially vary from other subjects who do not consume apples or other plant-based foods. We observe that in the cohort studies, we have done an analysis controlling for diet where we found the trends to be similar even after controlling for diet. In the randomized clinical trials, the assumption was made that the diets in the various arms were equivalent. Nevertheless, this substitution effect may be a limitation of this meta-analysis. While the majority of cohort studies controlled for diet and lifestyle variables in their analyses, the effect of dietary patterns or overall fruit intake may have confounded the associations from the few studies which did not control for these variables. Lastly, some RCTs failed to describe randomization techniques, varied in the types of controls used to compare the effects of apple intake, and failed to provide sufficient information regarding participants' compliance to the intervention.

## CONCLUSION

Except for a significant decrease in BMI, meta-analyses of apple or pear intake in RCTs did not show a statistically significant change in risk factors for CVD/CMD. Observational studies demonstrated an association between apple, pear, or combined apple and pear intake and a decreased risk of acute coronary disease, cerebrovascular disease, CVD mortality, diabetes and all-cause mortality suggesting that apple intake may be of benefit in CVD/CMD prevention.

## STATEMENT OF AUTHORS' CONTRIBUTIONS TO MANUSCRIPT

GR and EJJ: conceived, designed, and lead the project; BAG, EEA, EE, GR, and EJJ: screened articles and extracted data; EEA: performed the statistical analyses; NN: visualized data into forest plots; and BAG, EEA, NN, EJJ, and GR: wrote the manuscript and had primary responsibility for the final content. BAG, EEA, EE, NN, EJJ, and GR: read and approved of the final manuscript.

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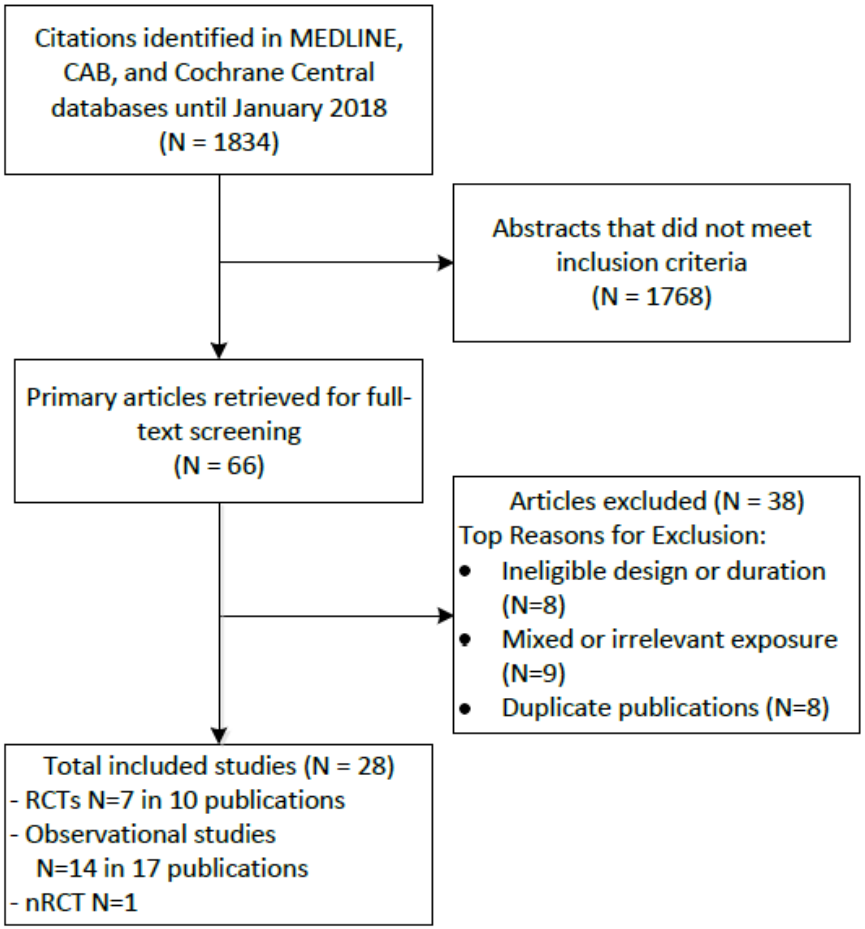

Figure 1: Results of comprehensive literature search

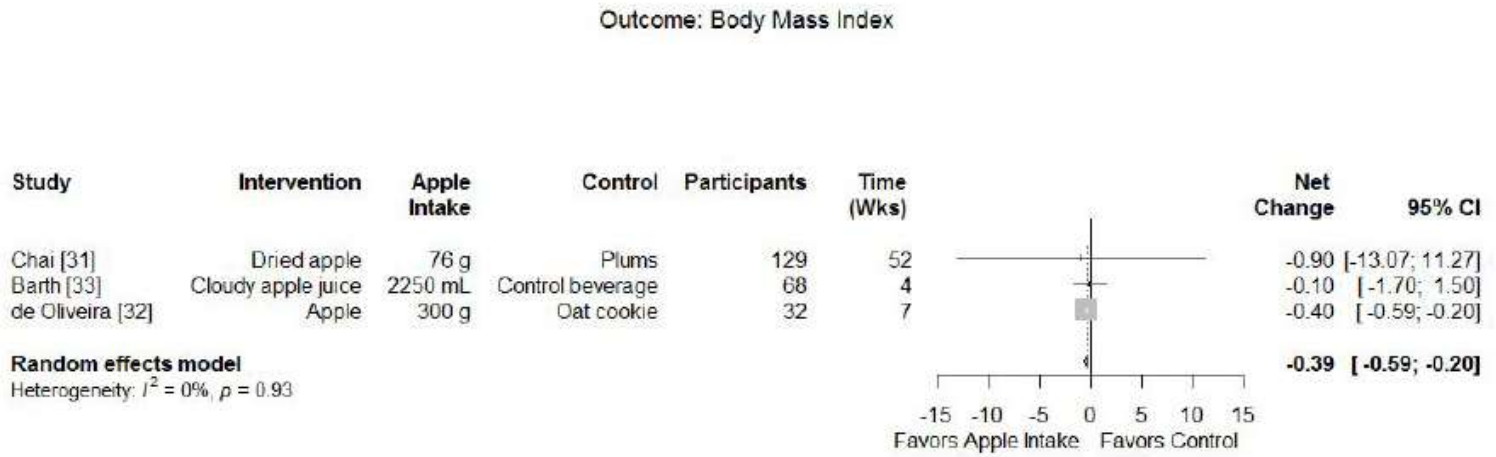


Figure 2: Meta-analysis of Intervention Trials Reporting the Effect of Apple versus Control on Body Mass Index

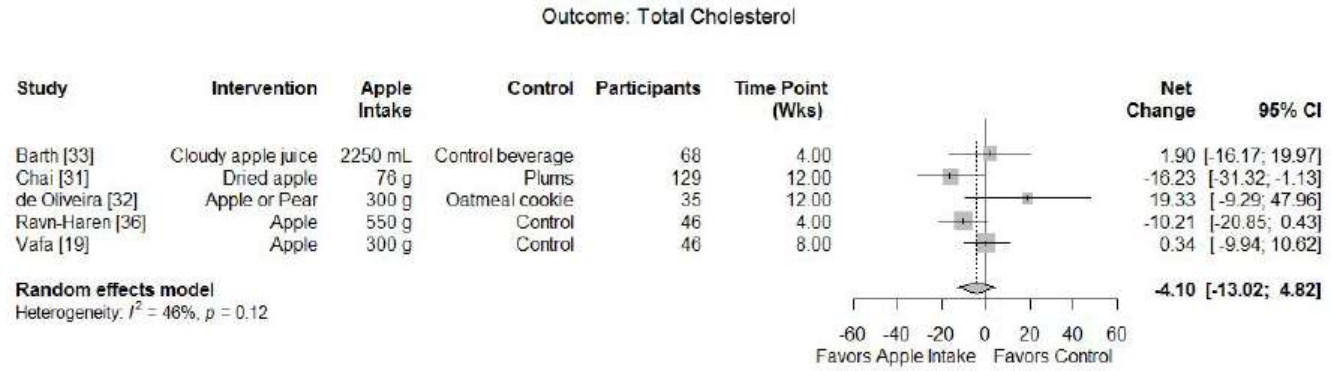


Figure 3: Meta-analysis of Intervention Trials Reporting the Effect of Apple versus Control on Total Cholesterol

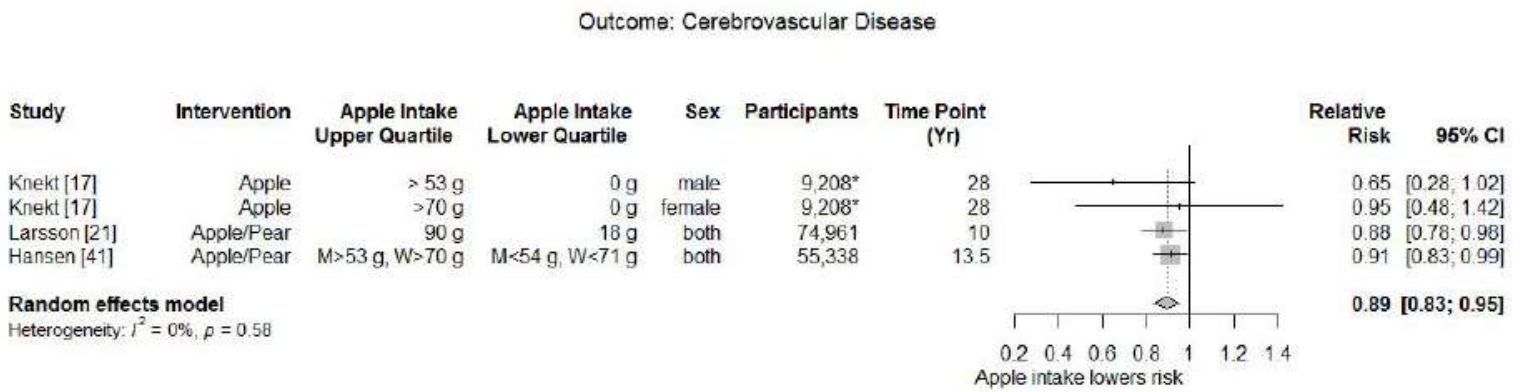


Figure 4: Meta-analysis of Observation Studies Reporting the Effect of Apple versus low dose on Cerebrovascular Disease

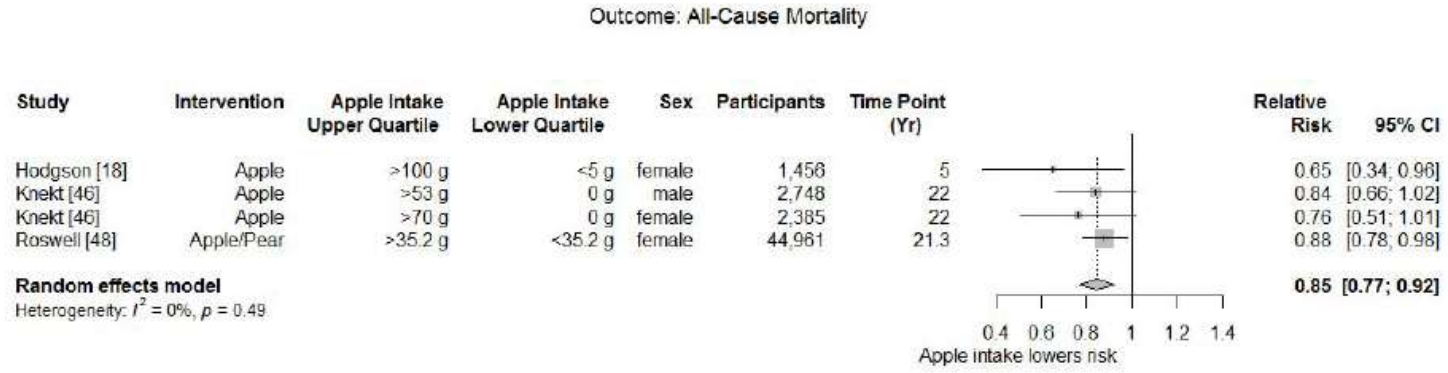


Figure 5: Meta-analysis of Observational Studies Reporting the Effect of Apple versus low dose on All-cause mortality

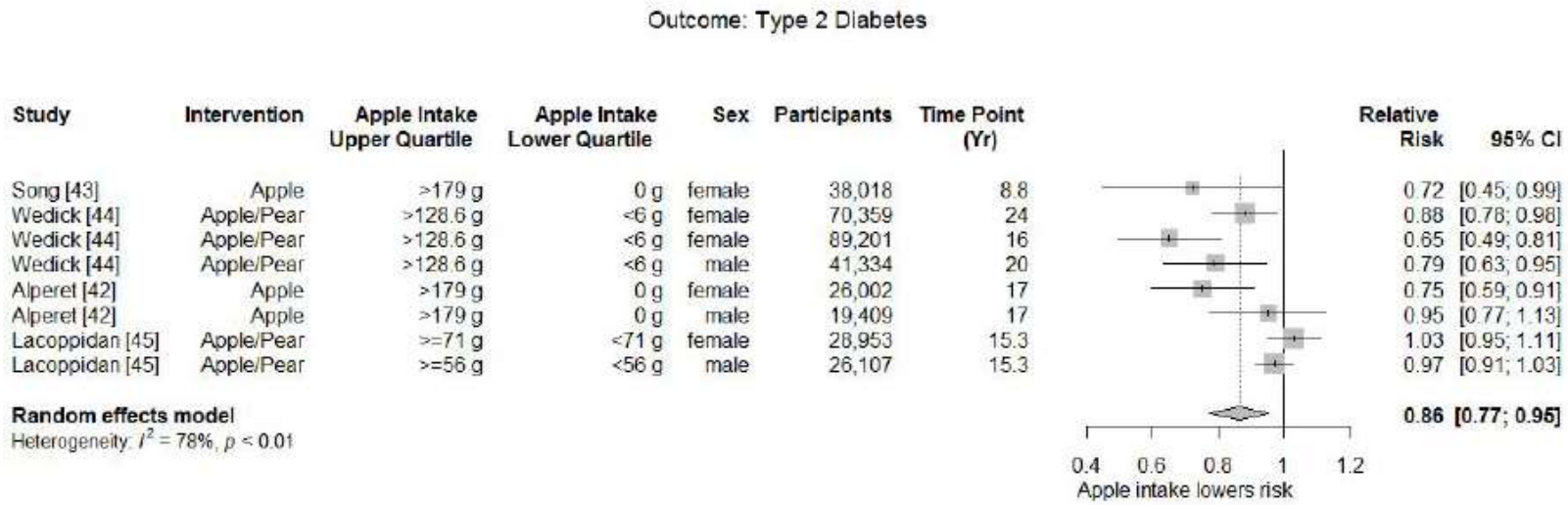


Figure 6: Meta-analysis of Observational Studies Reporting the Effect of Apple versus low dose on Type 2 Diabetes

**Table 1.** Baseline Study and Participant Characteristics of Intervention studies

| Author Year | Country (Funding source) | Study design (Duration) | N enrolled (N analyzed) | Male % | Mean age, y | BMI, kg/m$^2$ | Co-morbidities (%) | Apple/Pear, daily dose | Diet comparison, daily dose | Outcomes |
|---|---|---|---|---|---|---|---|---|---|---|
| Barth 2012 | Germany (NR) | RCT-P (4 weeks) | 68 (68) | 100 | 49 | 30.84 | Healthy: NR<br>Diabetes: 0<br>Hyperlipidemia/dyslipidemia: NR<br>Hypertension: NR<br>Existing CVD: NR | Apple juice, 2250 mL | Controlled beverage, 2250 mL | BMI, Body weight, HDL, LDL, TC, TG, Waist Circumference |
| Chai 2012; Hooshmand 2013 | United States (G) | RCT-P (1 year) | 160 (90) | 0 | 56.65 | 24.5 | Healthy: 100<br>Diabetes: 0<br>Hyperlipidemia/dyslipidemia: 0<br>Hypertension: 0<br>Existing CVD: 0 | Dried apple, 75 g | Dried plums, 100 g | BMI, Body weight, HDL, LDL, LDL/HDL, TC, TC/HDL, TG |
| De Oliveira 2003, 2008 | Brazil (G, A, I) | RCT-P (12 weeks) | 51 (35) | 0 | 44.03 | 31.87 | Healthy: 0<br>Diabetes: 0<br>Hyperlipidemia/dyslipidemia: 100<br>Hypertension: NR<br>Existing CVD: NR | Apple or pear, 900 g | Oatmeal cookie, 180 g | Blood glucose, BMI, body weight, glucose, glucose:insulin, insulin, TC, TG |

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|---|---|---|---|---|---|---|---|---|---|---|
| Gormley 1977 | Ireland (I) | nRCT-P (16 weeks) | 80 (76) | 100 | NR | NR | Healthy: NR<br>Diabetes: NR<br>Hyperlipidemia/dyslipidemia: NR<br>Hypertension: NR<br>Existing CVD: NR | Two additional apples per day to their diet | No more than 3 apples or apple-like fruits a week | TC, HDL |
| Navaei 2017; Johnson 2016 | NR (Fresh Pear Committee; Pear Bureau Northwest) | RCT-C (12 weeks) | 50 (43; 36) | NR | NR | NR | Healthy: 0<br>Diabetes: NR<br>Hyperlipidemia/dyslipidemia: NR<br>Hypertension: NR<br>Existing CVD: NR | Pear, 178 g/day | 50 g pear-flavored drink mix placebo | SBP, DBP, Waist:Hip |
| Ravn-Haren 2013 | Denmark (G) | RCT-C (20 weeks) | 23 (23) | 39 | 36.2 | 22.3 | Healthy: 100<br>Diabetes: 0<br>Hyperlipidemia/dyslipidemia: 0<br>Hypertension: 0<br>Existing CVD: 0 | Whole apple, 500 g; Cloudy apple juice, 500 mL; Clear Apple juice, 500 mL; Apple pomace, 22 mg | Only a restricted diet was allowed | Body weight, DBP, HDL, Insulin, LDL, SBP, TC, TC/HDL-C, TG, Waist-to-hip ratio |
| Svendsen 2015 | Norway (F) | RCT-P (8 weeks) | 118 (115) | 42.37 | 55 | 26 | Healthy: 0<br>Diabetes: NR<br>Hyperlipidemia/dyslipidemia: NR<br>Hypertension: 100<br>Existing CVD: 0 | Apple, 170 g/day | Green Kiwi fruit, 360 g | Body weight, DBP, SBP |
| Vafa 2011 | Iran (A) | RCT-P (8 weeks) | 46 (46) | 100 | 41.37 | 26.87 | Healthy 0<br>Diabetes: 0 | | No apple, 0 g | Apo B, HDL, |

| | | | | |
|---|---|---|---|---|
| | | Hyperlipidemia/dyslipidemia: 100<br>Hypertension: NR<br>Existing CVD: 0 | Apple, 300 g/day | LDL, LDL/HDL, Lp(a), TC, TG, VLDL |

A: Academic; Apo B: Apolipoprotein B; BMI: Body Mass Index; CVD: Cardiovascular Disease; DBP: Diastolic Blood Pressure; F: Foundation; G: Government; HDL: High Density Lipoprotein; I: Industry; LDL: Low Density Lipoprotein; Lp(a): Lipoprotein(a); NR: Not Reported; RCT-P: Randomized Control Trial-Cross-over; RCT-C: Randomized Control Trial- Parallel; SBP: Systolic Blood Pressure; TC: Total Cholesterol; TG: Triglycerides; VLDL: Very Low Density Lipoprotein. Note that Chai 2012 and Hooshmand 2013 as well as De Oliveira 2003 and De Oliveira 2008 were included as one row each in the above table since they drew data from the same trial.

**Table 2.** Meta-analysis of Intervention Trials Reporting the Effect of Apple versus Control on Serum Lipids and Body Composition

| | **N Studies (N Subjects)** | **Analysis 1 Net change (95%CI)** | **Analysis 2 Net change (95%CI)** | **Analysis 3 Net change (95%CI)** |
|---|---|---|---|---|
| **Total Cholesterol (mg/dL)** | 5 (324) | -4.10 (-13.02, 4.82) $I^2$ = 46.0% | -5.14 (-16.31, 6.03) *$I^2$ = 64.7%* | -4.50 (-14.45, 5.45) *$I^2$ = 54.8%* |
| **LDL-C (mg/dL)** | 4 (289) | -4.75 (-10.40, 0.90) $I^2$ = 19.0% | -7.09 (-15.54, 1.35) *$I^2$ = 61.4%* | -5.29 (-11.77, 1.18) $I^2$ = 34.4% |
| **HDL-C (mg/dL)** | 4 (289) | -0.79 (-2.58, 0.99) $I^2$ = 0.0% | -0.97 (-2.76, 0.82) $I^2$ = 0.0% | -0.75 (-2.55, 1.05) $I^2$ = 0.0% |
| **Triglycerides (mg/dL)** | 5 (324) | 8.91 (-10.32, 28.14) *$I^2$ = 59.1%* | 9.66 (-9.05, 28.36) *$I^2$ = 54.0%* | 8.60 (-14.93, 32.13) *$I^2$ = 72.4%* |
| **Body Weight (kg)** | 5 (393) | 0.14 (-0.45, 0.73) $I^2$ = 0.0% | 0.14 (-0.45, 0.73) $I^2$ = 0.0% | 0.14 (-0.45, 0.73) $I^2$ = 0.0% |

| | | |
|---|---|---|
| **BMI (kg/m$^2$)** | 3 (229) | **-0.39 (-0.59, -0.20)** **$I^2$ = 0.0%** |

Meta-analyses were conducted using the random-effects model.
Analysis 1: For one 3-arm parallel trial Chai 2012, the main analyses included 3 month intake time-point because it was the closest to the final time points in the other studies.
Analysis 2: For one 3-arm parallel trial Chai 2012, sensitivity analysis was conducted using the 6 month time point
Analysis 3: For one 3-arm parallel trial Chai 2012, sensitivity analysis was conducted using the 12 month time point
$I^2$ is an indicator of between-comparison heterogeneity. $I^2$ >50% was deemed as having significant heterogeneity.
BMI = Body Mass Index; HDL-C = High-density lipoprotein cholesterol; LDL-C = Low-density lipoprotein cholesterol; N = number; N/A = not applicable.

**Table 3.** Baseline Study and Participant Characteristics of Cohort studies

| Author, Year | Country (Funding source) | Cohort name | Duration (Enrollment year) | N enrolled (N analyzed) | Male, % | Mean age, y | BMI, kg/m$^2$ | Co-morbidities (%) | Apple, dose levels | Outcomes | Model adjustments |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Alperet, 2017 | United States, China (G, A) | Singapore Chinese Health Study | 1993-1998 (494,741 person-years= 17 years) | 63,257 (45,411) | 31 | Men: 57; Women: 56 | Men: 22.7; Women: 23.2 | Healthy: NR<br>Diabetes: 0<br>Hyperlipidemia/dyslipidemia: NR<br>Hypertension: 19.2<br>Existing CVD: 0 | Never/rarely; <1 serving/ week; 1 serving/ week; 2-3 serving/ week; 4-6 servings/ week; >1 serving/ day | Type 2 diabetes | Diet |
| Mink 2007; Arts, 2001 | United States (F,G) | None | 1986 (12 years) | 34,492 (34,492) | 0 | 61 | 26.9 | Healthy: 89.6-90.7<br>Diabetes: 5.7-6.5<br>Hyperlipidemia/dyslipidemia: NR<br>Hypertension: 34.1-34.8<br>Existing CVD: 0 | *Apples and pears:* <1 serving/ week; 1 serving/ week; >1 serving/ week | CHD mortality, CVD mortality, Stroke mortality | Age, alcohol intake, cholesterol, Diet |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bertoia, 2015 | United States (G) | NHS I; NHS II; HPFS | NHS: 1976; NHS II: 1989; HPFS: 1986 (4 years) | 133,468 (133,468) | 14.48 | 41.65 | NR | Healthy: NR Diabetes: 0 Hyperlipidemia/dyslipidemia: NR Hypertension: NR Existing CVD: 0 | *Apples and pears:* 0 servings/day; 0.31 servings/day | Body weight | Age, BMI, diet, smoking status, hours of sitting or watching TV, hours of sleep physical activity |
| Borgi, 2016 | USA (G, A) | NHS I; NHS II; HPFS | NHS: 1976; NHS II: 1989; HPFS: 1986 (2,939,124 person-years) | 187,453 (123,059) | 19.6 | 25-75 | NR | Healthy: NR Diabetes: NR Hyperlipidemia/dyslipidemia: NR Hypertension: 0 Existing CVD: NR | *Apples and Pear:* <1 per month; 1-3 per month; 1-3 per week; >4 per week | Hypertension | Age, alcohol intake, analgesic use, BMI, current oral contraceptive use, ethnicity, hypertension family history, menopausal status, physical activity smoking status, use, weight change |
| Hansen, 2010 | Denmark (G) | Danish Diet, Cancer and Health cohort | 1993-1997 (7 years) | 53,383 (53,383) | 46.95 | *Median*: Males 55; Females 56 | *Median*: Males 26; Females 25 | Healthy: NR Diabetes: 0 Hyperlipidemia/dyslipidemia: NR Hypertension: NR Existing CVD: 0 | *Men*: < 18g apple/day; 18 - 98g apple/day; > 98g apple/day *Women*: < 18g apple/day; 18 - 54g apple/day; > 54g apple/day | Acute Coronary Syndrome | BMI, diet, education level, smoking, alcohol intake, alcohol abstainers and physical activity, total cholesterol, systolic blood pressure |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Hansen, 2017 | Denmark (F) | Danish Diet, Cancer and Health cohort | 1993-1997 (Median: 13.5 years) | 57,053 (55,338) | 48 | *Mean of median*: 56.1 | Median: 25.5 | Healthy: NR<br>Diabetes: NR<br>Hyperlipidemia/dyslipidemia: NR<br>Hypertension: NR<br>Existing CVD: NR | *Apple and pear*:<br>Male:<br>0-56 g/d,<br>56< g/d<br>Females:<br>0-71 g/d,<br>71< g/d | Total stroke, ischemic stroke, hemorrhagic stroke, and intracerebral hemorrhage | Alcohol intake, BMI, diabetes, diet, education, hypertension, hypercholesterolemia. physical activity, smoking, atrial fibrillation, waist circumference |
| Hertog, 1993 | The Netherlands (G) | Zutphen Elderly Men study | 1985 (5 years) | 805 (805) | 100 | 71.26 | 25.5 | Healthy: NR<br>Diabetes: NR<br>Hyperlipidemia/dyslipidemia: NR<br>Hypertension: NR<br>Existing CVD: 37.3 | 0 apples/day;<br>0-1 apples/day; >1 apple/day | Cardiovascular mortality | Age, BMI, diet, and risk factors [includes history of MI in 1985, physical activity, smoking, serum TC and HDL, SBP] |
| Hodgson, 2016 | Australia (G, F, H) | Calcium Intake Fracture Outcome Study | 1998 (15 years) | 1500 (1456) | 0 | 75.16 | 27.2 | Healthy: NR<br>Diabetes: 6<br>Hyperlipidemia/dyslipidemia:18.52<br>Hypertension: 43.2<br>Existing CVD: 23.27 | <5 g/d;<br>5-100 g/d;<br>>100 g/d | CVD mortality | Age, alcohol intake, antihypertensive meds, BMI, cancer, cholesterol lowering meds, CVD, diabetes, diet, low-dose aspirin, physical activity smoking status, socio-economic status, treatment code |
| Knekt, 1996 | Finland (None) | Finnish mobile clinic | 1967- 1972 (20-23 years) | 5133 (5133) | 53.5 | 44.97 | 25.85 | Healthy: NR<br>Diabetes: NR<br>Hyperlipidemia/dyslipidemia: NR<br>Hypertension: 38.47<br>Existing CVD: 0 | Men:<br>>54g; 0 g;<br>Women:<br>>71g; 0 g | Coronary mortality; Total mortality | Age, BMI, cholesterol, hypertension, smoking status |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Knekt, 2000 | Finland (None) | Finnish mobile clinic | 1967 (28 years) | 9208 (9208) | NR | 37.46 | NR | Healthy: NR<br>Diabetes: NR<br>Hyperlipidemia/dyslipidemia: NR<br>Hypertension: NR<br>Existing CVD: 0 | Men: >54g; 0 g; Women: >71g; <5 g | Acute stroke; All cerebrovascular disease; Intracerebral haemorrhage; Thrombosis embolia | Age, BMI, diet, diabetes, geographical region, hypertension, serum cholesterol, smoking, occupation |
| Lacoppidan 2015 | Denmark (F, G) | Danish Diet, Cancer and Health cohort | 1993-1997 (Median: 15.3 years) | 57,053 (55,060) | 47.2 | *Median*: Males 55; Females 56 | *Median*: Males 24.8; Females 26.1 | Healthy: NR<br>Diabetes: 0<br>Hyperlipidemia/dyslipidemia: NR<br>Hypertension: NR<br>Existing CVD: NR | *Apple and pear*: Male: 0-56 g/d, 56< g/d Females: 0-71 g/d, 71< g/d | Type 2 Diabetes | Age, alcohol intake, BMI, diet, education level, physical activity, smoking, waist circumference, |
| Larsson, 2013 | Sweden (G) | SMC; COSM | 1998-2008 (10.2 years) | 74,961 (74,961) | 53.9 | 60.3 | NR | Healthy: NR<br>Diabetes: 5.96<br>Hyperlipidemia/dyslipidemia: NR<br>Hypertension: 20.8<br>Existing CVD: 0 | *Apple and pear*: 18 g/day; 36 g/day; 90 g/day; 180 g/day | Cerebral Infarction, Intracerebral hemorrhage, Subarachnoid hemorrhage, Total Stroke | Age, alcohol intake, aspirin use, BMI, diabetes, education, family history of myocardial infarction, history of hypertension, sex, smoking status and pack-years of smoking, physical activity |
| Oude Griep, 2011 | The Netherlands (G, I) | MORGEN Study | 1993-1997 (10.3 years) | 20,069 (20,069) | 45 | 42 | 24.85 | Healthy: 100<br>Diabetes: 0<br>Hyperlipidemia/dyslipidemia: 0<br>Hypertension: 0<br>Existing CVD: 0 | *Apple and pear*: 25 g/day increase in intake | Stroke | Age, alcohol intake, BMI, diet, educational level, family history of AMI, hormone replacement therapy use, sex, smoking status |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Roswell, 2015 | Sweden (G) | Swedish Women's Lifestyle and Health cohort | 1991- 1992 (21.3 years) | 44,961 (44,961) | 0 | 39 | 23.0 | Healthy: NR<br>Diabetes: 0<br>Hyperlipidemia/dyslipidemia: NR<br>Hypertension: NR<br>Existing CVD: 0 | *Apples and pears*:<br>< 35.2 g/day;<br>>= 35.2g/day | Total Mortality | Age, alcohol intake, BMI, current tobacco consumption, diet, education, smoking status, time since smoking cessation |
| Song, 2005 | USA (G) | Women's Health Study | 1993 (8.8 years) | 38,018 (38,018) | 0 | 53.88 | 25.8 | Healthy: NR<br>Diabetes: 0<br>Hyperlipidemia/dyslipidemia: NR<br>Hypertension: 25.08<br>Existing CVD: 0 | 0/week;<br><1/week;<br>2-6/week;<br>>1/day | Type 2 diabetes | Age, alcohol use, BMI, diet, exercise, family history of diabetes history of cholesterol, history of hypertension, smoking |
| Wedick, 2012 | United States (G) | NHS I; NHS II; HPFS | NHS (1984); NHS II (1991); HPFS (1986) (24 years) | NHS: 70,359; NHS II: 89,201; HPFS: 41,334 (3,645,585 person-years) | NHS: 0; NHS II: 0; HPFS: 100 | NHS: 50; NHS II: 36; HPFS: 53 | NR | Healthy: NR<br>Diabetes: 0<br>Hyperlipidemia/dyslipidemia: NR<br>Hypertension: 16.77<br>Existing CVD: 0 | <1 time/mo;<br>1-3 times/mo;<br>1 time/week;<br>2-4 times/week;<br>>5 times/week | Type 2 diabetes | Age, BMI, diet, ethnicity, family history of diabetes, hormone use, oral contraceptive use, postmenopausal status, smoking status |

AMI: Acute Myocardial Infarction; BMI: Body Mass Index; CHD: Coronary Heart Disease; COSM: Cohort of Swedish Men; CVD: Cardiovascular Disease; F: Foundation; G: Government; HDL: High Density Lipoprotein; H: Hospital Research Committee; HPFS: Health Professional Follow-up Study; I: Industry; MORGAN: Monitoring Project on Risk Factors and Chronic Diseases in the Netherlands; MI: Myocardial Infarction; NHS: Nurses' Health Study; NR: Not Reported; SBP: Systolic Blood Pressure; SMC: Swedish Mammography Cohort; TC: Total Cholesterol y; NR: Not Reported; SBP: Systolic Blood Pressure; SMC: Swedish Mammography Cohort; TC: Total Cholesterol

**Table 4.** Meta-analysis of Observation Studies Reporting the Effect of Apple versus low dose on CVD risk factors and events

| | N Studies (N Subjects) | Analysis 1 High dose | Analysis 1 Net change (95%CI) | Analysis 2 High dose | Analysis 2 Net change (95%CI) | Analysis 3 High dose | Analysis 3 Net change (95%CI) | Analysis 4 High dose | Analysis 4 Net change (95%CI) |
|---|---|---|---|---|---|---|---|---|---|
| **Cerebrovascular disease/ Total stroke** | 3 (139,507) | Knekt 2000, Hansen 2017 (M>=54g, W>=71g); Larsson (90g) | **0.89 (0.83, 0.95)** $I^2$ = 0.0% | Knekt 2000, Hansen 2017 (M>=54g; W>=71g); Larsson (180g) | **0.89 (0.83, 0.95)** $I^2$ = 3.5% | | | | |
| **Cardiovascular death** | 3 (36,753) | Hertog 1993 (>110g), Hodgson 2016 (>100g), Mink (>20 g) | **0.86 (0.78, 0.95)** $I^2$ = 0.0% | Hertog 1993 (19-110g), Hodgson 2016 (5-100g), Mink (>20 g) | **0.87 (0.79, 0.96)** $I^2$ = 0.0% | | | | |
| **Thrombosis or embolia or CI** | 2 (84,169) | Knekt 2000 (M>=54g, W>=71g); Larsson 2013 (90 g) | 0.76 (0.55, 1.05) $I^2$ = 50.4% | Knekt 2000 (M>=54g; W>=71g); Larsson 2013 (180 g) | 0.76 (0.55, 1.06) $I^2$ = 52.3% | | | | |
| **Type 2 Diabetes Incidence** | 4 (339,383) | Alperet 2017, Lacoppidan 2015(>56, >71), Song 2005 (>=180); Wedick (>128.6) | **0.86 (0.77, 0.95)** $I^2$ = 78.0% | Alperet 2017 (51-77g), Lacoppidan 2015(>56, >71), Song 2005 (51.4-154.3g); Wedick (51.4- 102.9g) | **0.88 (0.80, 0.96)** $I^2$ = 84.2% | Alperet 2017, Lacoppidan 2015(>56, >71), Song 2005 (<=25.7g); Wedick (25.7g) | **0.92 (0.86, 0.99)** $I^2$ = 74.9% | Alperet 2017, Lacoppidan 2015(>56, >71), Song 2005 (<=25.7g); Wedick (6-18g) | **0.93 (0.87, 0.99)** $I^2$ = 69.14% |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Type 2 Diabetes Incidence (Females-only)** | 3 (293,867) | Alperet 2017, Lacoppidan 2015( >71), Song 2005 (>=180); Wedick (>128.6) | **0.81 (0.68, 0.96)** $I^2$ = 89.7% | Alperet 2017 (51-77g), Lacoppidan 2015( >71), Song 2005 (51.4-154.3g); Wedick (51.4- 102.9g) | **0.86 (0.74, 0.98)** $I^2$ = 87.9% | Alperet 2017, Lacoppidan 2015( >71), Song 2005 (<=25.7g); Wedick (25.7g) | 0.92 (0.82, 1.02) $I^2$ = 81.34% | Alperet 2017, Lacoppidan 2015( >71), Song 2005 (<=25.7g); Wedick (6-18g) | 0.92 (0.83, 1.02) $I^2$ = 78.7% |
| **Intracerebral haemorrhage** | 3 (139,507) | Knekt 2000, Hansen 2017 (M>=54g, W>=71g); Larsson 2013 (90 g) | 0.93 (0.77, 1.12) $I^2$ = 0.0% | Knekt 2000, Hansen 2017 (M>=54g, W>=71g); Larsson 2013 (180 g) | 0.92 (0.76, 1.12) $I^2$ = 0.0% | | | | |
| **All-cause mortality** | 3 (51,550) | Knekt 1996 (M>=54g, W>=71g); Hodgson 2016 (>100 g); Roswell 2015 (>=35.2g) | **0.85 (0.77, 0.92)** $I^2$ = 0.0% | Knekt 1996 (M>=54g, W>=71g); Hodgson 2016 (5-100 g); Roswell 2015 (>=35.2g) | **0.85 (0.79, 0.92)** $I^2$ = 0.0% | | | | |

Meta-analyses were conducted using the random-effects model.
$I^2$ is an indicator of between-comparison heterogeneity. $I^2$ >50% was deemed as having significant heterogeneity.
N = number; N/A = not applicable.