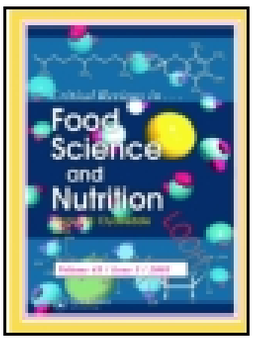

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Flavonoid subclasses and type 2 diabetes mellitus risk: a meta-analysis of prospective cohort studies

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Running title: Flavonoid subclasses and type 2 diabetes mellitus risk

Abstract: Epidemiological studies have suggested controversial associations between flavonoid subclasses and type 2 diabetes mellitus (T2DM) risk. The aim of the present meta-analysis was to quantitatively estimate these associations with prospective cohort study. A systematic literature search in PubMed and Scopus databases was performed up to May 2018. Multivariate-adjust relative risks (RRs) with corresponding 95% confidence intervals (CIs) for the highest versus the lowest category were pooled by using a random-effects model. Using restricted cubic spline regression model, non-linear dose-response analysis was estimated. Nine independent prospective cohort studies with 172,058 participants and 16910 events were included. Dietary intakes of

flavanols, flavonols, flavan-3-ols and isoflavones were inversely associated with T2DM risk, and the summary RRs were 0.86 (95%CI: 0.77, 0.97), 0.91 (95%CI: 0.85, 0.98), 0.90 (95%: 0.82, 0.99) and 0.91 (95%CI: 0.84, 0.98), respectively. Dose-response analysis showed that 135 mg/day increment of flavanols (95%CI: 0.92, 0.96; P for trend < 0.001), 50 mg/day increment of flavonols (95%CI: 0.88, 0.99, P for trend = 0.021), 68 mg/day increment of flavan-3-ols (95%CI: 0.92, 0.96, P for trend < 0.001), or 1.8 mg/day increment of isoflavones (95%CI: 0.92, 0.97, P for trend < 0.001) were associated with 6% reduction in T2DM risk. Non-significant association was observed with respect to flavanones and flavones. The present meta-analysis provides substantial evidence that dietary intakes of flavanols, flavonols, flavan-3-ols and isoflavones were inversely associated with T2DM risk, respectively. Higher dietary intakes of flavanol-, flavonol-, flavan-3-ol- and isoflavone-foods would have beneficial effects for protection against T2DM.

Keywords: Flavonoid subclasses; type 2 diabetes mellitus; prospective cohort study; meta-analysis

Introduction

Sufficient evidence supports plant-derived phytochemicals have beneficial effects protecting against non-communicable diseases, such as type 2 diabetes mellitus (T2DM), cardiovascular diseases and several cancers (Dillard and German, 2010; Guo et al., 2017; Knekt et al., 2002). Of these, flavonoids, which are the largest group of secondary metabolites in plants, have received widespread attention in scientific community. Flavonoids consist of two phenyl rings that are linked by an oxygenated heterocyclic ring. On the basis of their chemical structures, flavonoids are divided into six subclasses, including anthocyanins, flavanols or flavan-3-ols (flavan-3-ol

monomers, proanthocyanidins, and theaflavins), flavanones, flavones, flavonols, and isoflavones (Zamora-Ros et al., 2014).

T2DM is a major contemporary public issue because it is generally associated with diverse complications leading to premature mortality and morbidity (Bragg et al., 2017). Considering that the pathogenesis of T2DM is responsible for the interactions of genetics and environment, changes in lifestyles and dietary patterns have shown beneficial effects in the prevention of T2DM (Guo et al., 2016; Guo et al., 2017; Tuomilehto et al., 2001). A meta-analysis of prospective cohort studies has suggested that higher dietary intake of flavonoids were inversely associated with T2DM risk (Liu et al., 2014). Given various kinds of chemical structures, subclasses of flavonoids possess substantial differences of absorption, metabolism and bioavailability in human gastrointestinal tract and that might influence their bioactivities in the prevention of metabolic disorders (Manach et al., 2005; Williamson and Manach, 2005). It is therefore of important and necessary to clarify the subclasses of flavonoids associated with T2DM risk.

With the development of chromatography, the content of flavonoid subclasses was precisely assessed in plant foods. Up to now, a growing body of scientific research has reported the association of flavonoid subclasses with T2DM risk, and these conclusions have been inconsistent (Ding et al., 2016; Grosso et al., 2017; Kataja-Tuomola et al., 2011; Knekt et al., 2002; Song et al., 2005; Tresserra-Rimbau et al., 2016; Wedick et al., 2012; Zamora-Ros et al., 2013; Zamora-Ros et al., 2014). Only one prospective cohort study reported dietary intake of theaflavins associated with T2DM risk (Zamora-Ros et al., 2013), and two studies provided proanthocyanidins intake associated with T2DM risk (Tresserra-Rimbau et al., 2016; Zamora-Ros et al., 2013;). Besides, a previous meta-analysis reported that dietary intake of anthocyanins were

associated with T2DM risk (Guo et al., 2016). Thus, these flavonoid subclasses were excluded in the present study. Therefore, the present meta-analysis aimed to address the question of whether dietary intake of different flavonoid subclasses are associated with T2DM risk, including flavanols, flavonols, flavan-3-ols, isoflavones, flavanones, and flavones. Besides, dietary intakes of individual flavonoids associated with T2DM risk were also evaluated, including quercetin, keampferol and myricetin (Kataja-Tuomola et al., 2011; Knekt et al., 2002; Song et al., 2005; Zamora-Ros et al., 2013).

Methods

Search strategy and inclusive criteria

A systematic search for publications was conducted before May 2018 using PubMed and Scopus databases. Flavonoid, flavanol, flavan-3-ol, flavonol, flavanone, flavone, or isoflavone were paired with diabetes as search terms. Additionally, manual search was implemented to scrutinize publications from systematic reviews and meta-analyses, and original research. Inclusive criteria were prospective studies, including prospective cohort, nested case-control and case-cohort studies. The exposures of interest were any subtypes of flavonoids, and the endpoint of interest was incident of T2DM.

Data extraction and quality assessment

Two investigators independently implemented data extraction for identified publications. The extracted data included the characteristics of the studies, such as the surname of first author, publication year, region, duration of follow-up, mean age, and number of cases and participants. To eliminate the effects of covariates, multivariate-adjusted relative risk (RR) with the corresponding 95% confidence interval (CI) was extracted. During this period, any types of discrepancies were resolved through discussion.

Quality assessment was conducted on the basis of Newcastle-Ottawa scale (Wells et al., 2014). This scoring system was comprised of 9 aspects to each study: 4 for selection, 2 for comparability and 3 for assessment of outcomes. The maximum of score was 9 stars, and a study with stars of 0-3, 4-6, and 7-9 was classified as low, moderate and high quality, respectively.

Statistical analysis

Since the eligible studies used Cox proportional hazards models to evaluate the associations of flavonoid subclasses with T2DM risk, thus, the RR was identical to hazard ratio (HR) expressing their associations. To explore whether higher intakes of flavonoid subclasses were inversely associated with reduced risk of T2DM, the highest quantile versus the lowest quantile/reference was carried out for data synthesis. In this case, multivariate-adjusted RRs with the corresponding 95% CIs for the highest versus lowest category were log transformed and pooled by using a random-effects model as described by DerSimonian and Laird (DerSimonian and Laird, 1986). The studies, which contained 3 or more categories, were performed dose-response meta-analysis. The median dose of flavonoid subclass intake was extracted. If the median dose was not provided, the midpoint of the lower and upper categories was used as the dose of the quantile. The dose was defined as 1.2-fold of the highest boundary if the highest quantile was open-ended. A two-stage random-effects dose-response analysis was carried out to estimate the associations of flavonoid subclasses with T2DM risk. To estimate the potential curvilinear (non-linearity) associations between flavonoids subclasses and T2DM risk, a restricted cubic spline model was used with 3 knots at fixed percentiles of the flavonoid subclasses distribution (25%, 50% and 75%) (Harrell et al., 1988). A P value for potential curvilinear was calculated by testing the null hypothesis that the regression coefficient of the second spline was equal to zero (Orsini

et al., 2012). In the presence of substantial linear trend (P-value for curvilinear > 0.05), a linear dose-response meta-analysis was carried out for trend estimation by using generalized least squares regression as proposed by Greenland and Longnecker (Greenland and Longnecker, 1992) and Orsini, et al (Orsini et al., 2006) to assess the associations between increment of flavonoid subclasses and T2DM risk.

Heterogeneity among studies was estimated with I^2 statistic. The I^2 values of 25%, 50% and 75% as cut-off points indicate low, moderate and high degrees of heterogeneity. To examine the potential sources of heterogeneity, subgroup and univariate meta-regression analyses were implemented based on the information of these studies, including duration of follow-up (≤ 10 or > 10 years), region (Europe or U.S), study quality (moderate or high quality) and mean age of participants (≤ 50 or > 50 years). To evaluate whether any study exerted undue influence on summary estimate, sensitivity study was conducted by deleting one study at a time, and the pooled estimate was re-calculated. Begg's rank correlation test was used to assess publication bias (significant level at $P < 0.1$) (Egger et al., 1997). Statistical analysis was performed with STATA 11.0 for windows (Stata CORP, College station, TX). P-value was two-trailed with significant level of 0.05.

Results

Study selection

The process of study search is shown in Figure 1. After removal of 611 duplicate citations, 1597 unique articles were searched from PubMed and Scopus databases. After screening titles and abstracts, 1579 citations were excluded, leaving 18 articles for full-text examination. Of these, 9 citations were deleted because they did not meet

the inclusive criteria (e.g., study design or without reporting detailed data). Finally, 9 articles (9 independent prospective cohort studies) were eligible for data synthesis.

Study Characteristics

The characteristics of the studies are listed in Table 1. Two article consisted of the three independent cohort studies, namely, Nurses' Health Study (NHS), NHS II, and Health Professionals Follow-up Study (HPFS) (Ding et al., 2016; Wedick et al., 2012). Four prospective studies conducted in the U.S (Ding et al., 2016; Song et al., 2005; Wedick et al., 2012), and the others performed in Europe (Grosso et al., 2017; Kataja-Tuomola et al., 2011; Knekt et al., 2002; Tresserra-Rimbau et al., 2016; Zamora-Ros et al., 2013; Zamora-Ros et al., 2014). Among them, 4 independent prospective cohort studies reported the associations of individual flavonol (quercetin, kaempferol and myricetin) and flavone (apigenin and luteolin) intakes with T2DM risk (Kataja-Tuomola et al., 2011; Knekt et al., 2002; Song et al., 2005; Zamora-Ros et al., 2014), and 6 independent studies provided the associations between subclasses of flavonoids (flavanols, flavonols, flavanones or flavones) and T2DM risk (Grosso et al., 2017; Nettleton et al., 2006; Tresserra-Rimbau et al., 2016; Wedick et al., 2012; Zamora-Ros et al., 2013). Four independent studies provided the association of flavan-3-ols intake with T2DM risk (Wedick et al., 2012; Zamora-Ros et al., 2013), and five independent studies investigated the association of isoflavones intake and T2DM risk (Ding et al., 2016; Grosso et al., 2017; Zamora-Ros et al., 2013). Zamora-Ros et al., reported that flavonoid subclasses and individual flavonols associated with risk of T2DM, respectively (Zamora-Ros et al., 2013; Zamora-Ros et al., 2014). For the studies without reporting flavonols or flavones associated with T2DM risk, we combined RR of individual flavonols (quercetin, kaempferol and myricetin) and flavones (apigenin and luteolin) associated with T2DM risk by using a fixed-effects model, representing the associations of flavonols and flavones with

T2DM risk, respectively (Kataja-Tuomola et al., 2011; Knekt et al., 2002; Song et al., 2005). Based on Newcastle-Ottawa scale, 3 studies were classified as high-quality (Ding et al., 2016; Knekt et al., 2002; Wedick et al., 2012; Zamora-Ros et al., 2013), and the remaining studies were regarded as moderate-quality (Grosso et al., 2017; Kataja-Tuomola et al., 2011; Song et al., 2005; Tresserra-Rimbau et al., 2016) (Supplementary Table 1).

Subtypes of flavonoids and T2DM risk

Five independent cohort studies provided dietary intake of flavanols associated with T2DM risk (Grosso et al., 2017; Wedick et al., 2012; Zamora-Ros et al., 2013), and the pooled effect indicated that a higher flavanols intake was associated with 14% reduction of T2DM risk (95%CI: 0.77, 0.97), with a significant between study heterogeneity (I^2 = 74.1%, P = 0.004) (Figure 2). Nine independent prospective cohort studies reported the association of flavonols intake with T2DM risk (Grosso et al., 2017; Kataja-Tuomola et al., 2011; Knekt et al., 2002; Song et al., 2005; Tresserra-Rimbau et al., 2016; Wedick et al., 2012; Zamora-Ros et al., 2013). A higher intake of flavonols was inversely associated with risk of T2DM (Pooled RR = 0.91; 95%CI: 0.85, 0.98), with a significant between-study heterogeneity (I^2 = 53.4%, P = 0.028) (Figure 2). Four independent studies investigated the association of flavan-3-ols intake with T2DM risk (Wedick et al., 2012; Zamora-Ros et al., 2013), and a higher intake of flavan-3-ols was associated with 10% (95%CI: 0.82, 0.99) reduction of T2DM risk, with a significant between study heterogeneity (I^2 = 64.1%, P = 0.039) (Figure 3). The combined estimate showed that a higher intake of isoflavones was inversely associated with T2DM risk with five independent studies (Ding et al., 2016; Grosso et al., 2017; Zamora-Ros et al., 2013), and the pooled effect was 0.91 (95%CI: 0.84, 0.98), with no significant between study heterogeneity (I^2 = 10.6%, P = 0.346) (Figure 3). Seven independent cohort studies provided available

data with respect to flavones (Grosso et al., 2017; Song et al., 2005; Tresserra-Rimbau et al., 2016; Wedick et al., 2012; Zamora-Ros et al., 2013) and flavanones (Grosso et al., 2017; Knekt et al., 2002; Tresserra-Rimbau et al., 2016; Wedick et al., 2012; Zamora-Ros et al., 2013). The combined estimates showed that dietary intakes of flavanones (Pooled RR = 1.01; 95%CI: 0.93, 1.10) and flavones (Pooled RR = 0.97; 95%CI: 0.87, 1.08) were not associated with T2DM risk, respectively (Figure 4). Five prospective cohort studies were eligible for dose-response analysis, and significant curvilinear association was observed between flavanols intake and T2DM risk by using restricted cubic splines models (P for non-linearity = 0.003) (Figure 5) (Grosso et al., 2017; Wedick et al., 2012; Zamora-Ros et al., 2013). Dose-response analysis suggested that 135 mg/day increment of flavanols intake was associated with 6% reduction in T2DM risk (95%CI: 0.92, 0.96; P for trend < 0.001). Five independent cohort studies were available to investigate the association between flavonols intake and T2DM risk (Grosso et al., 2017; Wedick et al., 2012; Zamora-Ros et al., 2013). Non-significant curvilinear association was found (P for non-linearity = 0.081), but linear dose-response analysis indicated that 50 mg/day increment of flavonol intake was associated with 6% reduction in T2DM risk (95%CI: 0.88, 0.99, P for trend = 0.021) (Figure 5). Significant curvilinear association was observed between flavan-3-ols intake and T2DM risk (P for non-linearity = 0.012) (Wedick et al., 2012; Zamora-Ros et al., 2013), dose-response analysis showed that 68 mg/day increment of flavan-3-ols intake was associated with 6% reduction in T2DM risk (95%CI: 0.92, 0.96, P for trend < 0.001) (Figure 6). The pooled effect of the five independent studies indicated non-significant curvilinear association between isoflavones and T2DM risk (P for non-linearity = 0.679) (Ding et al., 2016; Grosso et al., 2017; Zamora-Ros et al., 2013). Dose-response analysis showed that 1.8 mg/day increment of isoflavones intake was associated with 6% reduction in T2DM risk (95%CI: 0.92, 0.97, P for trend < 0.001) (Figure 6).

Individual flavonols and T2DM risk

Four independent prospective cohort studies reported the associations of individual flavonols with T2DM risk (Kataja-Tuomola et al., 2011; Knekt et al., 2002; Song et al., 2005; Zamora-Ros et al., 2014). The pooled estimates suggested that higher dietary intakes of quercetin (Pooled RR = 0.94; 95%CI: 0.84, 1.04; I^2 = 0.0%), kaempferol (Pooled RR = 0.95; 95%CI: 0.87, 1.05; I^2 = 0.0%) and myricetin (Pooled RR = 0.90; 95% CI: 0.76, 1.05; I^2= 58.6%) were associated with a lower risk of T2DM, respectively, but the summary estimates did not research statistically significant difference (Supplementary Figure 1-3).

Publication bias and sensitivity analysis

Using Begg's rank correlation test, there was non-significant publication bias with respect to flavanols (P = 0.279), flavonols (P = 0.862), flavan-3-ols (P = 0.624), isoflavones (P = 0.806), flavanones (P = 0.302), flavones (P = 0.406), myricerin (P = 0.993), kaempherol (P = 0.599) and quercetin (P = 0.847). In sensitivity analysis, each study was sequentially excluded at a time, and the remaining data were not substantially driven with deletion of any one study (Supplementary Figure 4-12).

Subgroup analysis

Subgroup analysis stratified by mean age of participants indicated that the pooled effect of flavanols intake was significantly association with T2DM risk in participants with mean age > 50 years (Pooled RR = 0.77, 95%CI: 0.62, 0.96) (Table 2). The studies stratified by study quality indicated that the summary RR of flavonols intake was inversely associated with T2DM risk in high-quality studies (Pooled RR = 0.87, 95%CI: 0.81, 0.94). Meanwhile, there was a marginally significant difference with meta-regression between groups (P for meta-regression = 0.061) (Table 2). Similarly,

the summary estimate of flavan-3-ols intake was inversely associated with T2DM risk in participants with mean age > 50 years (Pooled RR = 0.90, 95%CI: 0.83, 0.99) (Table 3). The pooled effect of isoflavones intake was significantly associated with reduced risk of T2DM in studies with high quality (Pooled RR = 0.91, 95%CI: 0.83, 0.99) (Tables 3). However, non-significant difference was found between groups with meta-regression (Table 3). For dietary intake of flavanones, the pooled effect was significant in studies with moderate quality (Pooled RR = 0.72, 95%CI: 0.57, 0.91) (Table 4). Significant difference was found between groups with meta-regression (P for meta-regression = 0.027). Besides, there was a marginally significant difference in studies stratified by duration of follow-up (P for meta-regression = 0.051) (Table 4).

Discussion

The present study was the first to investigate the associations of flavonoid subclasses with T2DM risk. Based on the summary estimates, higher dietary intakes of flavanols, flavonols, flavan-3-ols and isoflavones were significantly associated with reduced risk of T2DM. Dose-response analysis suggested that 135 mg/day increment of flavanols, 50 mg/day increment of flavonols, 68 mg/day increment of flavan-3-ols and 1.8 mg/day increment of isoflavones were associated with 6% reduction in T2DM risk, respectively. Although intakes of myricerin, kaempherol and quercetin were associated with a lower risk of T2DM, the pooled effects did not research statistical difference.

Given the difficulty in separation and purification of flavonoids, few randomized controlled trials (RCTs) have investigated their effects in participants with T2DM. A 12 weeks cross-over trial showed that supplementation with soy protein and isoflavones significantly decreased the mean values of fasting insulin and insulin

resistance, compared with baseline in postmenopausal women with T2DM (Jayagopal et al., 2002). Another one year RCT with flavan-3-ols and isoflavones as intervention was carried out in postmenopausal women with T2DM, and the combined intervention showed a significant improvement in insulin sensitivity, and reduction in insulin concentrations, compared with the placebo group (Curtis et al., 2012). On the contrary, another cross-over or parallel RCTs with isoflavones as intervention have no favorable effects on glycemic control and insulin sensitivity in postmenopausal women with prediabetes (Hall et al., 2006; Liu et al., 2010). Besides, two months cross-over trial indicated that supplemental green tea-extract (456 mg catechins) showed a significant reduction in serum glycated hemoglobin (HbA1c) levels, but non-significant effect on fasting glucose concentrations in subjects with borderline diabetes (Fukino et al., 2008). Considering above-mentioned RCTs using different subtypes of flavonoids and duration as intervention, these studies could not obtain a consistent conclusion. In addition to RCTs, a cross-sectional study, which performed in Korean, suggested that dietary intake of flavones were significantly associated with a reduction in risk of T2DM (Oh et al., 2017).

To date, plenty of prospective cohort studies have estimated the association of flavonoid intake with T2DM risk, and the conclusions have been inconsistent. Recently, a meta-analysis of prospective cohort studies showed that a higher dietary intake of flavonoids was inversely associated with risk of T2DM (Liu et al., 2014). Considering that the subclasses of flavonoids present various chemical structures, these subtypes might have deferential anti-inflammatory and anti-oxidant effects to protect against T2DM. Therefore, we raised a hypothesis that several subtypes of flavonoids might be inversely associated with T2DM risk, whereas the other subtypes might be not. With the development of chromatography, the content of flavonoid subclasses in foods has been precisely measured in food items, and that provides available data to investigate the associations between subclasses of flavonoids and

T2DM risk. The conclusions from the present study indicated that higher dietary intakes of flavanols, flavonols, flavan-3-ols and isoflavones were inversely associated with T2DM risk, respectively, whereas non-significant association was found with respect to flavones and flavanones.

Low-grade chronic inflammation and oxidative stress impair pancreatic β-cell functions, and interfere with insulin signaling pathway, contributing to the initiation and development of T2DM. We summarized the possible mechanisms through which dietary intakes of flavanols/flavonols/flavan-3-ols/isoflavones protected against T2DM (Figure 7). 1) Anti-oxidative properties of flavonoids were associated with reduced risk of T2DM. Free radicals and reactive oxygen species (ROS), which are derived from normal oxygen metabolism and exogenous damage, can attract various inflammatory mediators and are associated with etiology and pathology of chronic diseases (Higdon and Frei, 2003). Based on the free hydroxyl groups, flavanols/flavonols/flavan-3-ols/isoflavones can scavenge free radicals directly through hydrogen or electron donating activity. The chemical reactions between flavanols/flavonols/flavan-3-ols/isoflavones and free radicals generate a more stable and less-reactive radicals (Nijveldt et al., 2001). The transition metal ions, such as iron and copper, are participated in metal-catalyzed formation of free radicals. Flavonoids, flavonols in particular, could inhibit metal-catalyzed free radical formation through iron-chelating and iron-stabilizing properties (Rice-Evans et al., 1997; Ferrali et al., 1997). In addition, increases in the activity of antioxidant enzymes have shown as an indirect anti-oxidative effect. Extensive animal models indicated that intervention with flavonoids significantly increased the concentrations of glutathione peroxidase (GPX), catalase, and superoxide dismutase (SOD) in blood and tissues, compared with control group (Khan et al., 1992; Lin et al., 1998). Xanthine oxidase pathway has been shown as a pivotal route in the oxidative injury to tissues in rodent model (Sanhueza., 1992). During ischemic conditions, the

configuration of xanthine dehydrogenase is transformed into xanthine oxidase, which reacts with molecular oxygen to generate superoxide free radicals (Nijveldt et al., 2001). It has been reported that flavonoals (quercetin and silibin) and flavanols (catechins) could inhibit the formation of ROS by inhibiting the activity of xanthine oxidase to prevent oxidative injury (Aucamp et al., 1997; Chang et al., 1993; Iio et al., 1986). 2) Flavanols/flavonols/flavan-3-ols/isoflavones are also participated in inflammatory signaling pathways to protect against T2DM. Chronic inflammation has been recognized as a key role in the initiation, propagation, and development of metabolic disorders (Baker et al., 2011). During the stage of stimuli, the release of transcription factors, such as nuclear factor (NF)-κB and activator protein (AP)-1, are able to translocate to the nucleus, and bind to DNA, resulting in overexpression of inflammatory cytokines. Substantial studies of cell and animal models indicated that treatment with flavonoids was found to inhibit the activity of NF-κB and AP-1 (Higdon and Frei, 2003). Long-chain polyunsaturated fatty acids can be catalyzed by lipoxygenases and cyclooxygenases to form leukotrienes and prostaglandins, which are associated with chronic inflammation. It has been reported that flavonoid treatment inhibited the activities of lipoxygenases and cyclooxygenases in rat peritoneal leukocytes (Laughton et al., 1991). 3) Flavanols/flavonols/flavan-3-ols/isoflavones might regulate expression levels of key genes and proteins involving in glucose and lipid metabolism to prevent T2DM. Nuclear receptors and transcription factors, including peroxisome-proliferator activated receptor (PPAR) family, sterol regulated element binding proteins (SREBPs) and liver X binding receptors (LXRs) are implicated in carbohydrate and lipid synthesis and oxidation responsible for metabolic disorders (Edwards et al., 2002; Guo et al., 2017). It has been reported that flavan-3-ols/isoflavones could regulate PPAR family, SREBPs and LXRs in favor of glucose and lipid metabolism (Babu et al., 2013; Mezei et al., 2003; Mezei et al., 2006). Besides, Flavonoid subclasses such as isoflavonols could stimulate the express levels of phosphorylation of AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC)

contributing to glucose uptake, fatty acid metabolism and oxidation, and insulin sensitivity (Cederroth et al., 2008; Lorentecebrián et al., 2009; Barros and Gustafsson, 2011). Through aforementioned mechanisms, flavanols/flavonols/flavan-3-ols/isoflavones favorably improve glucose and lipid metabolism and enzyme systems activities, have antioxidant, metal chelation, anti-inflammatory properties, and stimulate pancreatic β-cell proliferation and differentiation beneficial for insulin signaling pathway.

Several strengths of the study should be put forward. First, the present study was the first meta-analysis to quantitatively evaluate the associations between subclasses of flavonoids and T2DM risk. The conclusions from 9 independent cohort studies with large simple-size demonstrated substantial evidence that higher intakes of flavonols, flavanols, flavan-3-ols and isoflavones were inversely associated with T2DM risk, respectively. Second, the included studies were prospective cohort studies with moderate and high quality, which reduced the possibility of recall errors and selection bias. Third, sensitivity analysis indicated that the pooled RR was not driven after removal of any one study, indicating the stability of the conclusions. Besides, non-significant publication bias was found, suggesting that the unpublished studies or missing data would not influence the summary estimates. Simultaneously, several limitations of the study should be acknowledged. First, RCT is the best way to investigate supplemental flavonoid subclasses on risk factors of T2DM. Considering that few RCTs have conducted with subclasses of flavonoids as intervention, prospective cohort studies were included in the present study. The strength of prospective cohort study is that it provides a causal inference and reflects the real world of dietary intakes of flavonoid subclasses associated with T2DM risk. Second, the accuracy of flavonoid intake assessment and calculation is crucial for the component-based epidemiological approach. Imprecision of flavonoid evaluation, which is the foremost limitation of these studies, would influence the final

conclusions. Flavonoids are found in various plant-derived foods, including fruits, vegetables, grains, roots, and beverages. The dietary intakes of flavonoids might be under-estimated rather than over-estimated by validated FFQ. Third, although the multivariable-adjusted RRs were extracted for data synthesis, prospective cohort study was inevitably influenced by inherent or unmeasured confounding factors. Besides, although the underlying mechanisms could not explain exactly why intakes of flavanols, flavonols, flavan-3-ols and isoflavones were inversely associated with reduced T2DM risk, antioxidant and free radical scavenging activities of them are attributed to the number and position of free hydroxyl groups, through which act their hydrogen donating capability (Bobilya, 2002; Mora et al., 1990). Further investigation of the metabolism is warranted to explore the structure-activity relationships regarding flavonoid subclasses.

The findings of the present meta-analysis provides sufficient evidence that dietary intakes of flavanols (such as tea, wine, apple and dark chocolate), flavonols (such as apple, onion, cauliflower and cabbage), flavan-3-ols (such as cocoas, tea and wine) and isoflavones (soybeen and related products) are inversely associated with T2DM risk. Diet rich in flavanols and flavonols, flavan-3-ols and isoflavones should be chosen to reduce the risk of T2DM. Since the whole studies are performed in Western countries, further studies are warranted in other regions and ethnic origins to confirm these associations.

Conflicts of interest

None.

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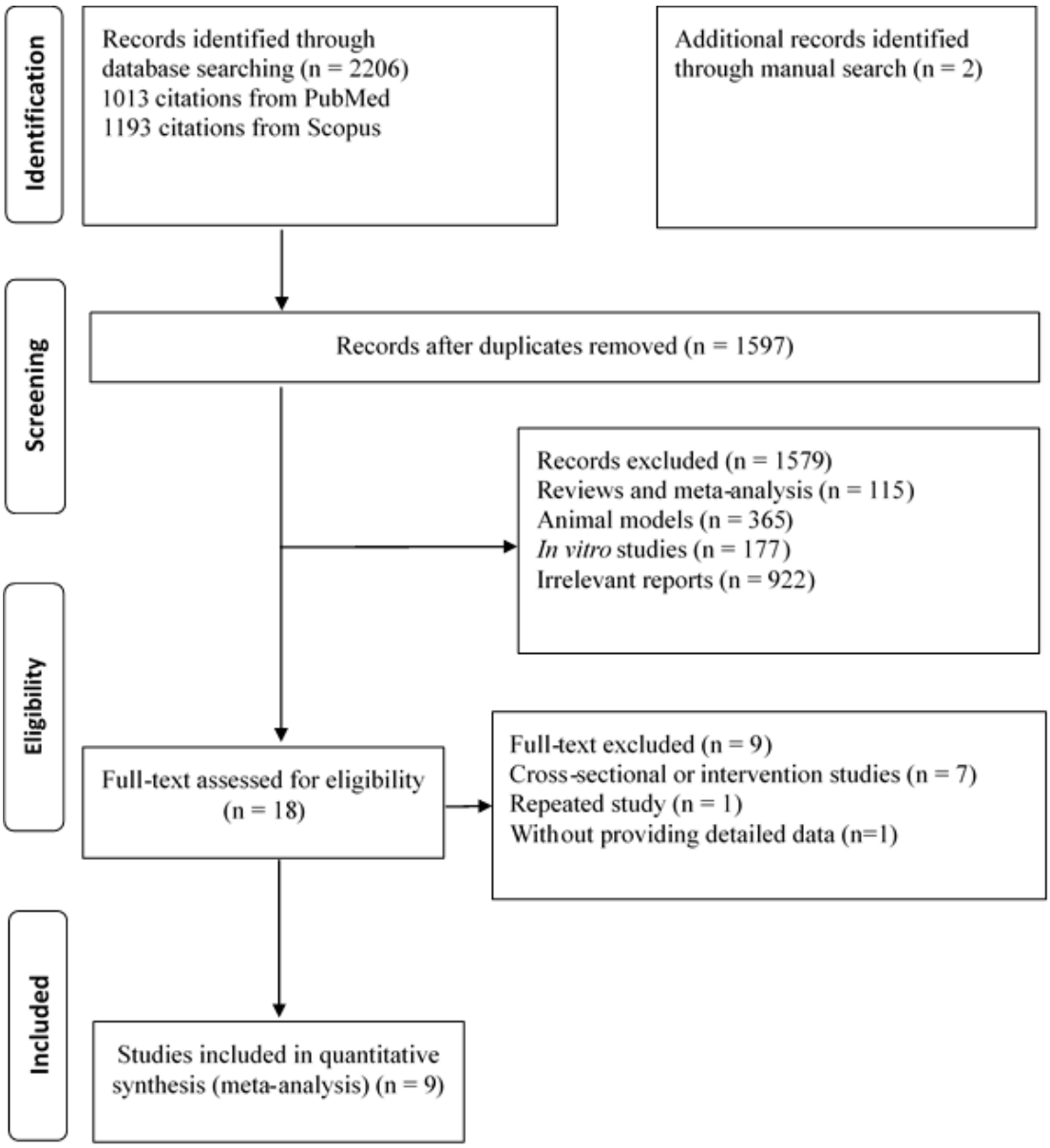

Figure 1. The process of study selection.

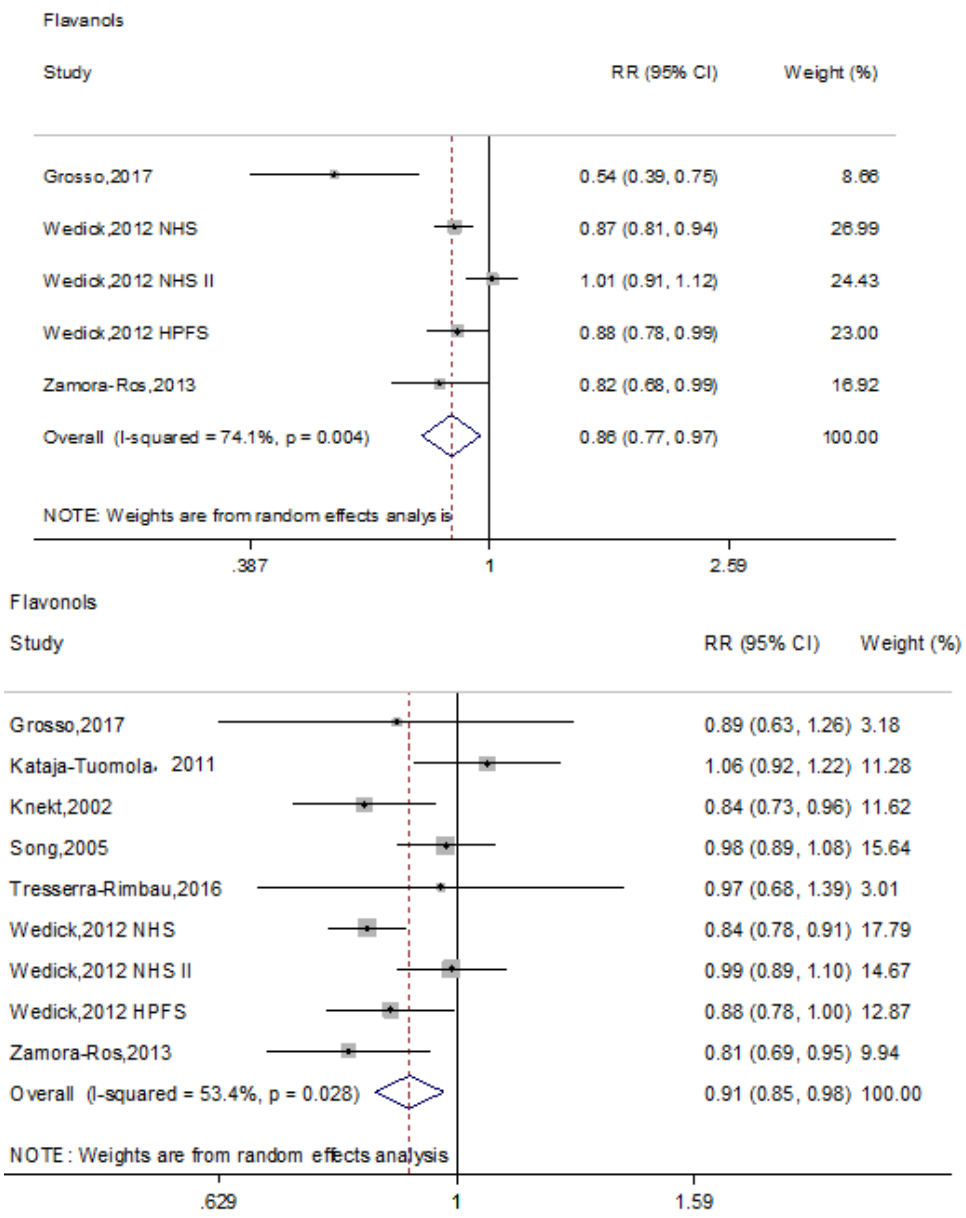

Figure 2. Forest plot to quantify the association between flavanols and flavonols and T2DM risk. The summary RR was calculated by using a random-effects model for the highest versus lowest category. RR, relative risk; T2DM, type 2 diabetes mellitus.

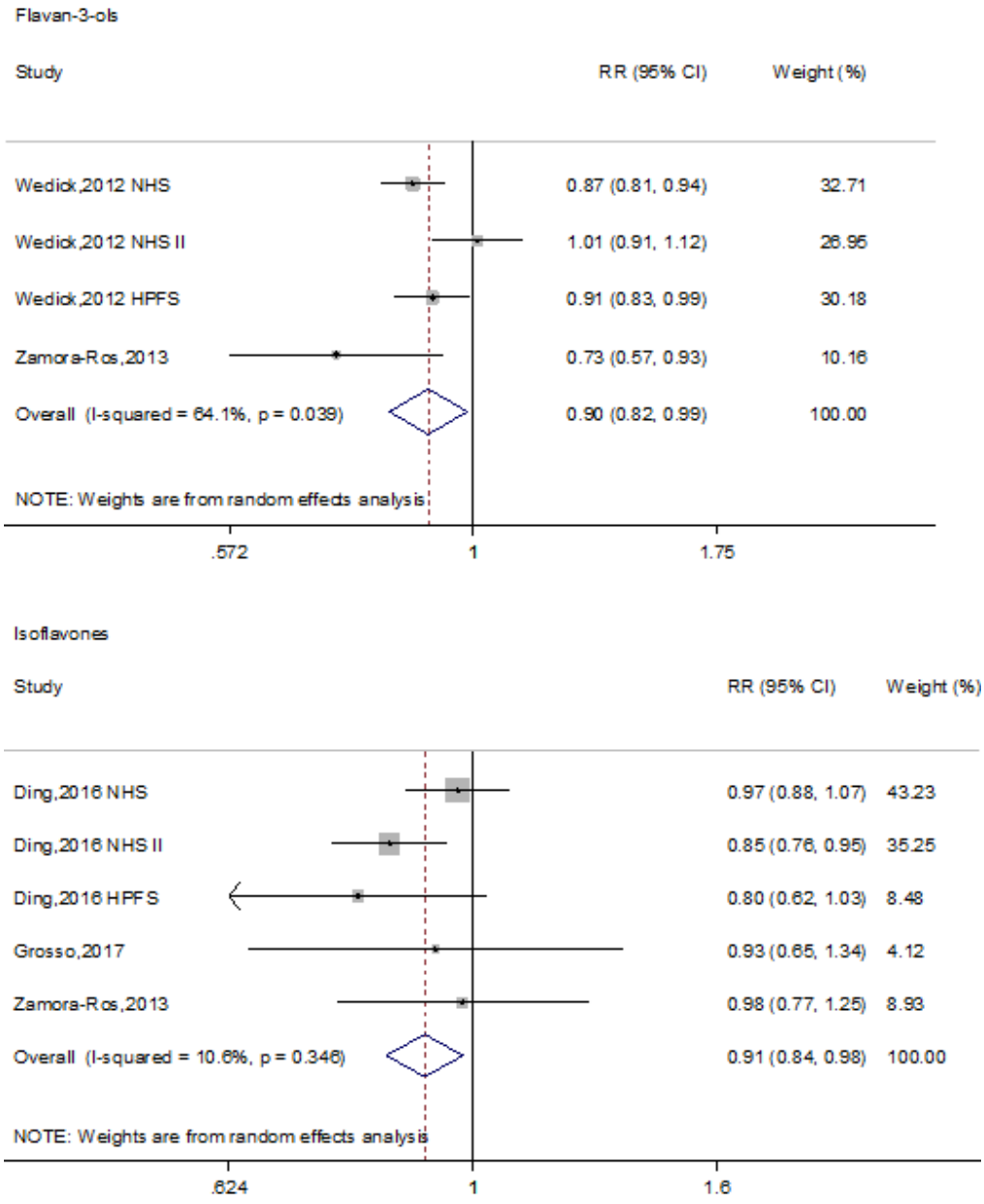

Figure 3. Forest plot to quantify the association between flavan-3-ols and isoflavones and T2DM risk. The summary RR was calculated by using a random-effects model for the highest versus lowest category. RR, relative risk; T2DM, type 2 diabetes mellitus.

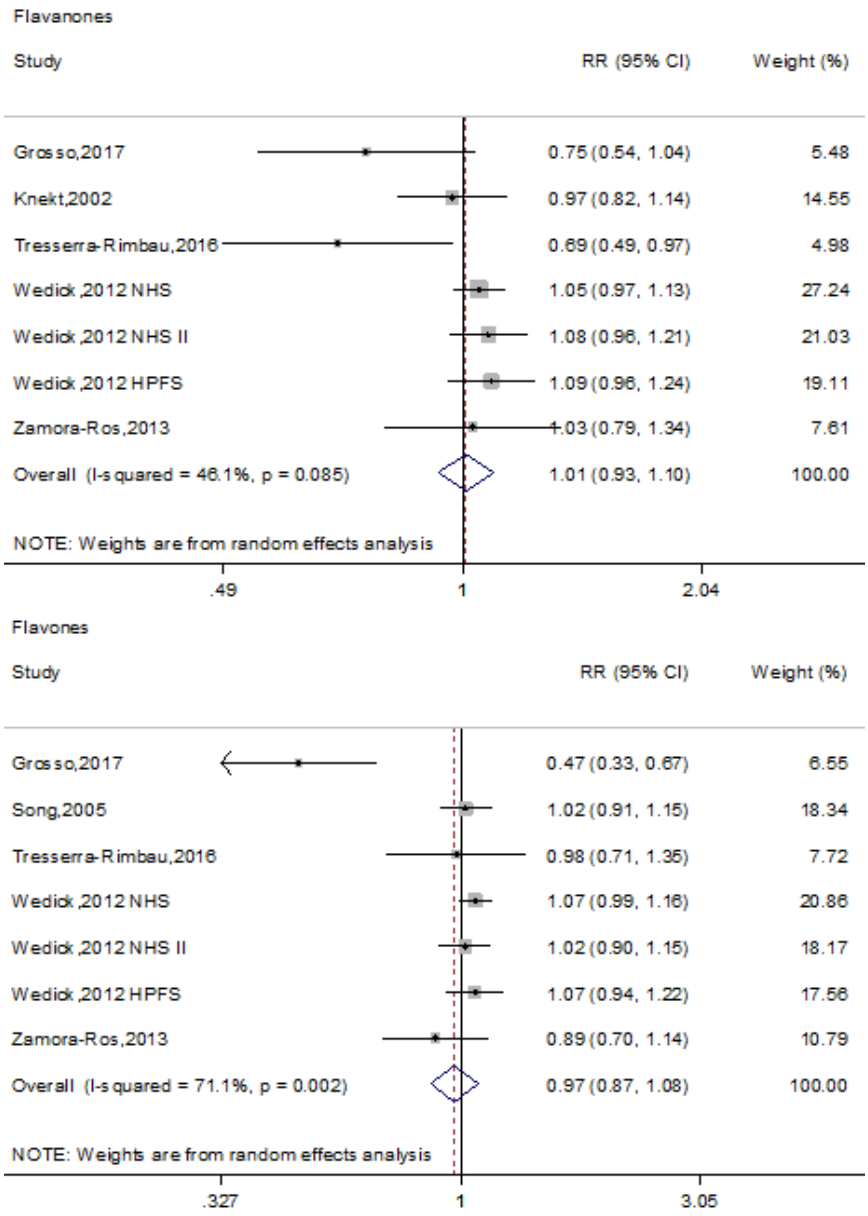

Figure 4. Forest plot to quantify the association between flavanones and flavones and T2DM risk. The summary RR was calculated by using a random-effects model for the highest versus lowest category. RR, relative risk; T2DM, type 2 diabetes mellitus.

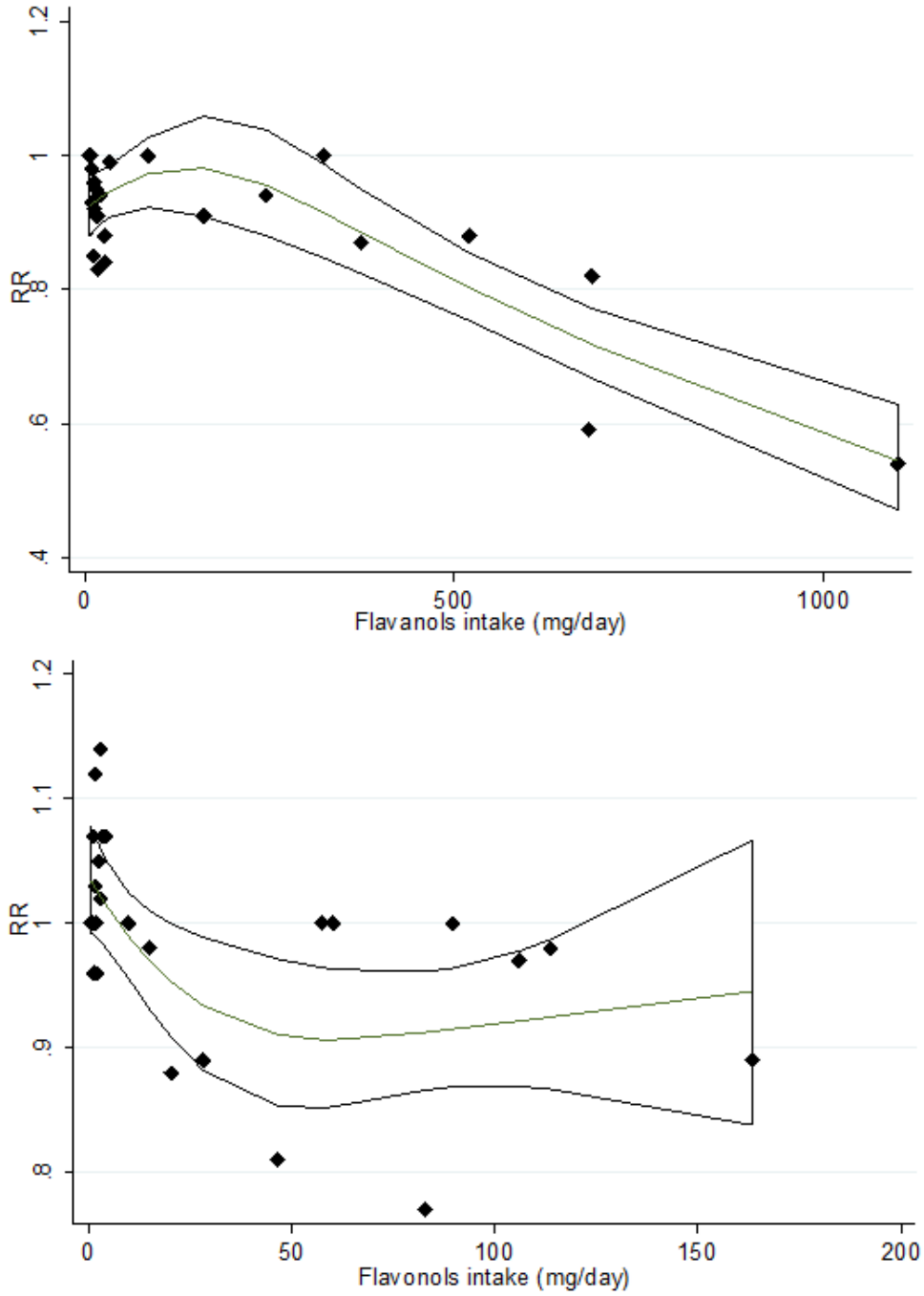

Figure 5. Dose-response analysis for the curvilinear association between intakes of flavanols and flavonols and T2DM risk. RR, relative risk; T2DM, type 2 diabetes mellitus.

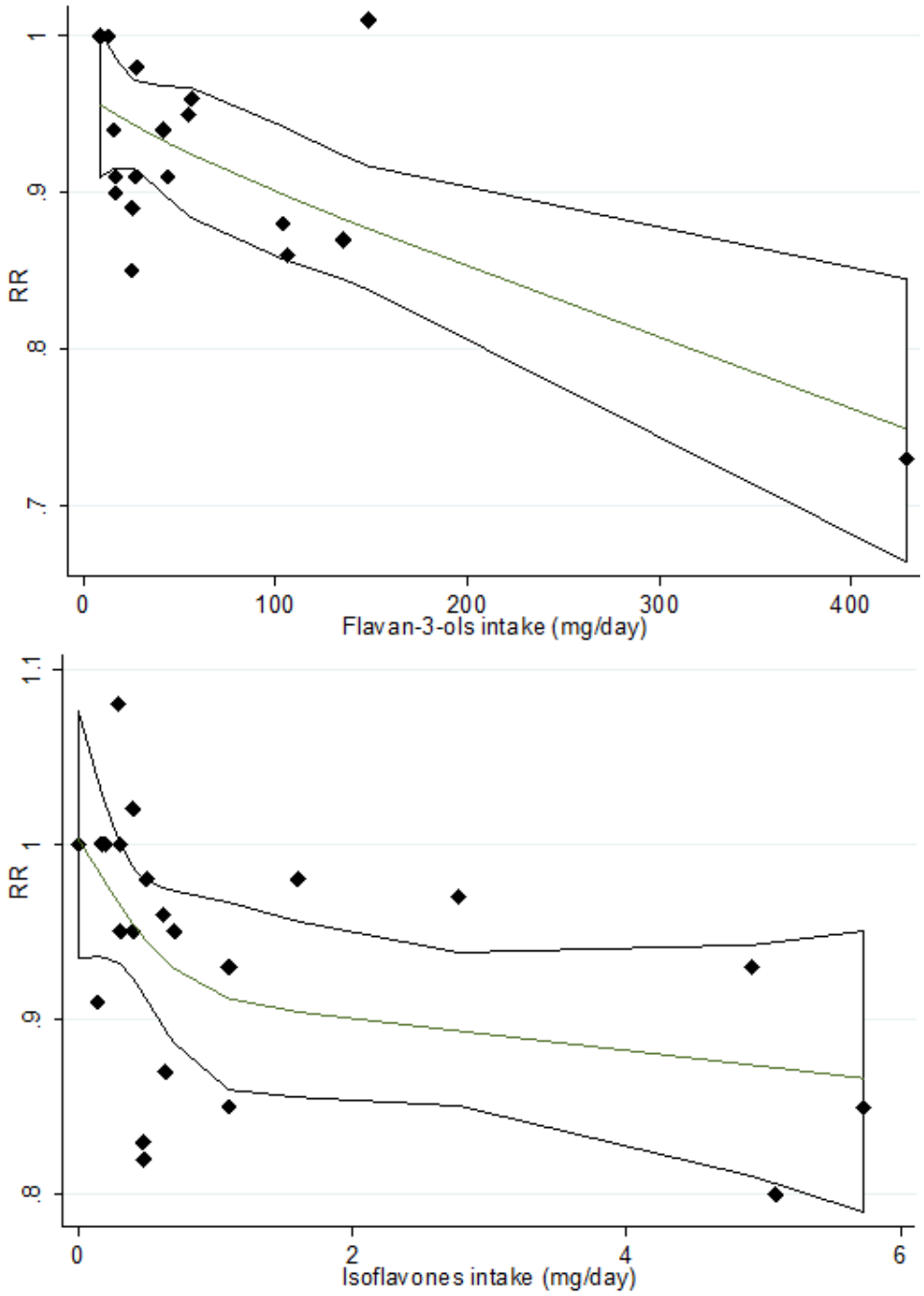

Figure 6. Dose-response analysis for the curvilinear association between intakes of flavan-3-ols and isoflavones and T2DM risk. RR, relative risk; T2DM, type 2 diabetes mellitus.

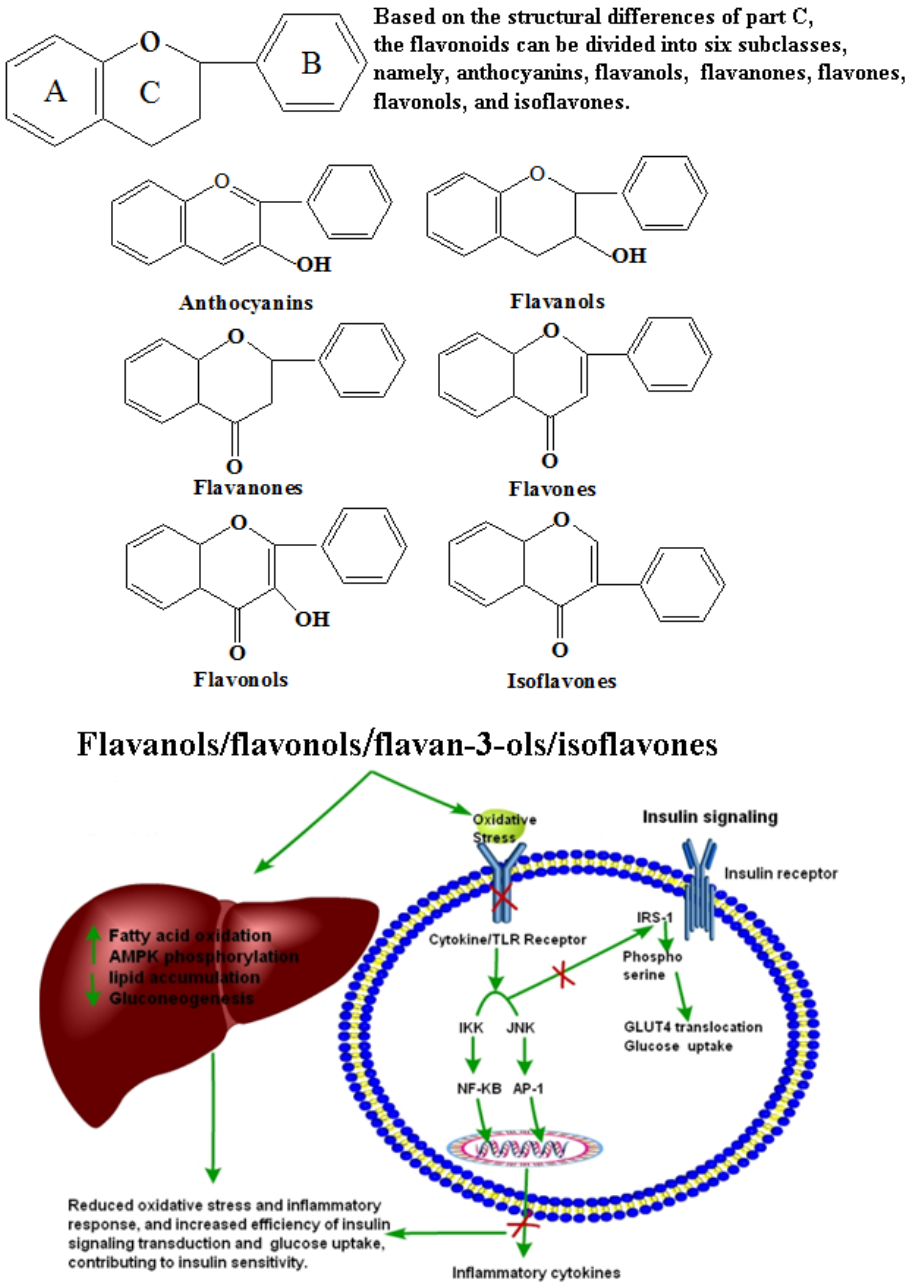

Figure 7. The possible mechanisms of flavanols/flavonols/flavan-3-ols/isoflavones protecting against T2DM. Oxidative stress results in the activation of cytokine and TLR pathways. JNK and IKK are participated in relaying stress signals throughout the cell and engaging metabolic responses. These kinases can inhibit insulin signaling via serine phosphorylation of IRS-1, finally blocking insulin action downstream of receptor activation. Through anti-oxidative bioactivities, flavanols/flavonols/flavan-3-ols/isoflavones might inhibit oxidative stress and the productions of reactive oxygen species. Accordingly, the transcription factors, such as AP-1 and NF-κB, which can upregulate the transcription of inflammatory genes, are inhibited. Meanwhile, flavanols/flavonols/flavan-3-ols/isoflavones lead to insulin action downstream of

receptor activation, contributing to insulin sensitivity and glucose uptake. Abbreviations: AP-1, activator protein-1; IKK, inhibitor of NF-κB kinase; IRS-1, insulin receptor substrate 1; GLUT4, glucose transporter 4; NF-κB, nuclear factor-κB; TLR, toll-like receptor.

Table 1. The characteristics of included prospective cohort studies

First author and cohort	Publication year and region	Age (gender)	Subjects (cases)	Duration of follow-up	Exposure measure	Outcome measure	Exposure	Covariates adjusted
Ding, NHS	2016, U.S	64 (F)	63,115 (4,519)	14 y	USDA flavonoid content of the foods database	National Diabetes Data Group criteria	Isoflavones	Age, race, family history of T2D, baseline disease status, BMI, physical activity, overall dietary pattern, total energy intake, smoking status. Menopausal status and postmenopausal hormone use were further adjusted for in women.
NHS II		45 (F)	79,061 (3,920)	12 y				
HPFS		67 (M)	21,281 (742)	8 y				

Grosso, HAPIEE	2017, Polish	57.4 ± 6.9	5806 (456)	4 y	Phenol-Explorer database	Self-reported	Flavanols Flavonols Flavanones Flavones Isoflavones	Age, total energy intake, BMI, physical activity, educational status, smoking status, alcohol consumption, alcohol intake, menopausal status and dietary fiber
Kataja-Tuomola	2011, Finland	57.7 ± 5.1 (M)	29, 133 (660)	10.2 y	Composition analyses from the Netherlands	Physician	Kaempferol, Myricetin, Quercetin	Age, supplementation, BMI, smoking status, smoking years, blood pressure, total cholesterol, high-density lipoprotein cholesterol, leisure-tim

								e physical activity and daily intake of alcohol and energy
Knekt	2002, Finland	39.3 ± 15.8	10,054 (526)	7 y	Flavonoid database	Physician	Quercetin Kaempferol Myricetin	Sex and age
Song, WHS	2005, U.S	≥ 45 y (F)	38,018 (1,614)	8.8 y	Food tables maintained by the Department of Nutrition, Harvard School of Public Health, Boston	Self-reported diagnoses	Apigenin Luteolin Quercetin kaempferol Myricetin	Age, BMI, total energy intake, smoking, exercise, alcohol, history of hypertension, history of high cholesterol, and family history of diabetes, fiber intake, glycemic load, magnesium and total

								fat
Tresserra-Rimbau	2016, Spain	66.6 ± 6.0	3430 (314)	5.51 y	Phenol-Explorer database	American Diabetes Association criteria	Flavanones Flavones Flavonols	Age, sex, recruitment center, intervention group, smoking, BMI, physical activity, dyslipidemia, hypertension, education level, total energy intake, alcohol intake, adherence to the Mediterranean diet, and glucose levels
Wedick,	2012, U.S				USDA flavonoid content of the foods database	National Diabetes Data Group criteria	Flavonols Flavones Flavanones	Age, BMI, smoking, alcohol intake, multivitamin use, physical activity, history of
NHS		49.9 ± 7.1 (F) 36.2 ± 4.7	70,359 (6,878) 89,201	24y				
NHS II				16y				
HPFS				22y				

		(F) 52.8 ± 9.5 (M)	(3,084) 41334 (2,649)					diabetes, postmenopausal status and hormone use, oral contraceptive use, ethnicity, total energy, intakes of red meat, fish, whole grains, coffee, high-calorie sodas, and trans fat
Zamora-Ros, EPIC	2013, Europe	52.4 ± 9.1	15,258 (729)	17 y	USDA databases, Phenol-Explorer and the U.K. Food Standards Agency database	Self-report, linkage to primary, secondary, and medication registers, hospital admissions, and mortalit	Flavanols Flavonols Flavan-3-ols Flavanones Flavones Isoflavones	Age, sex, total energy intake, educational level, physical activity, smoking, BMI, alcohol intake, intakes of red meat, processed

						y data		meat, sugar-sweetened soft drinks, coffee, intakes of fiber, vitamin C, and magnesium
Zamora-Ros, EPIC	2013, Europe	52.4 ± 9.1	15,258 (729)	17 y	USDA databases, Phenol-Explorer and the U.K. Food Standards Agency database	Self-report, linkage to primary, secondary, and medication registers, hospital admissions, and mortality data	Quercetin Kaempferol Myricetin	Age, sex, total energy intake, educational level, physical activity, smoking, BMI, alcohol intake, intakes of red meat, processed meat, sugar-sweetened soft drinks, coffee, intakes of fiber, vitamin C, and

magnesium

Abbreviations: EPIC, the European Prospective Investigation into Cancer and Nutrition; HAPIEE, the Health, Alcohol and Psychosocial factors in Eastern Europe; NHS, Nurses' Health Study; NHS II, Nurses' Health Study II; HPFS, Health Professionals Follow-up Study; USDA, the United States Department of Agriculture; WHS, Women's Health Study.

Table 2. Subgroup analysis of flavanols and flavonols associated with type 2 diabetes mellitus

Factors stratified	Flavanols					Flavonols				
	No.	Pooled effect (95%)	Heterogeneity		P^b	No.	Pooled effect (95%)	Heterogeneity		P^b
			I^2 (%)	P^a				I^2 (%)	P^a	
Mean age, years					0.342					0.497
≤ 50	2	0.93 (0.81, 1.08)	74.1	0.004		3	0.89 (0.79, 0.99)	70.0	0.035	
> 50	3	0.77 (0.62, 0.96)	72.7	0.026		6	0.93 (0.86, 1.02)	37.7	0.155	
Duration, years					0.071					0.926
≤ 10	1	0.54 (0.39, 0.75)	0.0	1.00		4	0.92 (0.84, 1.01)	14.8	0.318	
> 10	4	0.90(0.83, 0.98)	54.6	0.085		5	0.91 (0.83, 1.00)	69.5	0.011	
Region					0.202					0.831
Europe	2	0.68 (0.45, 1.02)	78.1	0.032		5	0.90 (0.80, 1.02)	50.4	0.089	
U.S	3	0.92 (0.83, 1.01)	64.1	0.062		4	0.92 (0.84, 1.00)	67.1	0.028	

Study quality				0.071					0.061
Moderate	1	0.54 (0.39, 0.75)	0.0	1.00		4	1.00 (0.92, 1.08)	0.0	0.732
High	4	0.90(0.83, 0.98)	54.6	0.085		5	0.87 (0.81, 0.94)	47.4	0.107

Abbreviations: CI, confidential interval; No., number of included studies; P^a, value for heterogeneity within subgroup; P^b, value for heterogeneity between subgroups with meta-regression analysis.

Table 3. Subgroup analysis of flavan-3-ols and isoflavones associated with type 2 diabetes mellitus

Factors stratified	Flavan-3-ols					Isoflavones				
	No.	Pooled effect (95%)	Heterogeneity		P^b	No.	Pooled effect (95%)	Heterogeneity		P^b
			I^2 (%)	P^a				I^2 (%)	P^a	
Mean age, years					0.518					0.220
≤ 50	2	0.93 (0.81, 1.08)	80.9	0.022		1	0.85 (0.76, 0.95)	0.0	1.00	
> 50	2	0.90 (0.83, 0.99)	63.8	0.096		4	0.95 (0.87, 1.03)	0.0	0.556	
Duration, years										0.520
≤ 10	0						0.84 (0.68, 1.03	0.0	0.502	
> 10	4	0.90 (0.82, 0.99)	64.1	0.039			0.92 (0.84, 1.02)	39.7	0.190	
Region					0.252					0.596
Europe	1	0.73 (0.57, 0.93)	0.0	1.00		2	0.96 (0.79, 1.18)	0.0	0.814	
U.S	3	0.92 (0.85, 1.00)	61.9	0.072		3	0.89 (0.80, 1.00)	51.1	0.129	

Study quality									0.903
Moderate	0				1	0.93 (0.65, 1.34)	0.0	1.00	
High	4	0.90 (0.82, 0.99)	64.1	0.039	4	0.91 (0.83, 0.99)	32.7	0.216	

Abbreviations: CI, confidential interval; No., number of included studies; P^a, value for heterogeneity within subgroup; P^b, value for heterogeneity between subgroups with meta-regression analysis.

Table 4. Subgroup analysis of flavanones and flavones associated with type 2 diabetes mellitus

Factors stratified	Flavanones					Flavones				
	No.	Pooled effect (95%)	Heterogeneity		P^b	No.	Pooled effect (95%)	Heterogeneity		P^b
			I^2 (%)	P^a				I^2 (%)	P^a	
Mean age, years					0.439					0.346
≤ 50	3	1.01 (0.93, 1.10)	0.0	0.576		3	0.97 (0.87, 1.08)	83.4	< 0.001	
> 50	4	0.91 (0.73, 1.13)	68.0	0.025		4	0.84 (0.62, 1.13)	0.0	0.745	
Duration, years					0.051					0.333
≤ 10	3	0.83 (0.66, 1.04)	52.6	0.121		3	0.80 (0.52, 1.23)	87.6	< 0.001	
> 10	4	1.06 (1.01, 1.12)	0.0	0.945		4	1.05 (0.99, 1.11)	0.0	0.517	
Region					0.078					0.094
Europe	4	0.88 (0.74, 1.05)	43.6	0.150		3	0.75 (0.50, 1.13)	81.1	0.005	
U.S	3	1.07 (1.01, 1.13)	0.0	0.854		4	1.05 (1.00, 1.11)	0.0	0.879	

Study quality				0.027					0.333
Moderate	2	0.72 (0.57, 0.91)	0.0	0.728	3	0.80 (0.52, 1.23)	87.6	< 0.001	
High	5	1.05 (1.00, 1.11)	0.0	0.837	4	1.05 (0.99, 1.11)	0.0	0.517	

Abbreviations: CI, confidential interval; No., number of included studies; P^a, value for heterogeneity within subgroup; P^b, value for heterogeneity between subgroups with meta-regression analysis.