REVIEW

Effect of Flavonoids on Upper Respiratory Tract Infections and Immune Function: A Systematic Review and Meta-Analysis[1,2]

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ABSTRACT

Previous research on animals indicates flavonoid compounds have immunomodulatory properties; however, human research remains inconclusive. The aim of this systematic review was to assess the efficacy of dietary flavonoids on upper respiratory tract infections (URTIs) and immune function in healthy adults. A created search strategy was run against Cochrane Central Register of Controlled Trials, MEDLINE, EMBASE and EMBASE classic, CINAHL, and AMED. The returned studies were initially screened, and 2 reviewers independently assessed the remaining studies for eligibility against prespecified criteria. Fourteen studies, of 387 initially identified, were included in this review, and the primary outcome measure was the effect of flavonoids on URTI incidence, duration, and severity. Of the included studies, flavonoid supplementation ranged from 0.2 to 1.2 g/d. Overall, flavonoid supplementation decreased URTI incidence by 33% (95% CI: 31%, 36%) compared with control, with no apparent adverse effects. Sick-day count was decreased by 40% with flavonoid supplementation, although unclear. Differences in bio-immune markers (e.g., interleukin-6, tumor necrosis factor-α, interferon-γ, neutrophils) were trivial between the intervention and control groups during the intervention and after exercise when a postintervention exercise bout was included. These findings suggest that flavonoids are a viable supplement to decrease URTI incidence in an otherwise healthy population. *Adv Nutr* 2016;7:488–97.

Keywords: flavonoids, quercetin, upper respiratory tract infection, common cold, systematic review, meta-analysis, immune function, exercise, polyphenols

Introduction

Upper respiratory tract infections (URTIs)[5] include an array of acute illnesses that affect the upper respiratory system, including sinusitis, tonsillitis, pharyngitis, otitis media, laryngitis, and the "common cold" (1). URTIs are a common health problem in society; 23.1% of a population of US adults reported a URTI in a 4-wk period, which resulted in a loss of 5.9 h work/wk per URTI episode (2). In general, adults have 2 or 3 URTIs each year, whereas children can average up to 5 (3). URTI symptoms usually appear and peak 24–72 h after infection but can last up to 7–14 d (3–5). More than 200 viruses are reported to cause URTIs, which can be classified into 7 categories, each with different seasonal and viral rates: orthomyxoviruses, parainfluenza, coronaviruses, paramyxoviruses (respiratory syncytial virus; RSV), herpes virus, adenoviruses, and picornaviruses (6). Bacterial URTIs alone are rare with <10% of total causation; however, coinfection with viruses may occur (7, 8).

The current treatment options for the common cold are typically symptom related, but currently there is no effective cure. The first-line treatment for the common cold is rest, fluids, maintenance of hydration status, and prevention of viral/bacterial spread (3). Antibiotics are ineffective to treat viral infections, but analgesics and antipyretics can be prescribed and purchased over the counter to relieve symptoms such as pain and/or fever (3).

URTIs are also a common health problem in athletes, as reported in several studies (9–12). Engebretsen et al. (9, 10) completed 2 retrospective studies on the 2010 Winter and 2012 Summer Olympics. In both studies, Engebretsen et al. (9, 10) found that 6.7% and 7.1%, respectively, of athletes reported an illness that was mostly respiratory related. This trend is also seen when people are training constantly. Nieman et al. (13) showed that 40% of the study participants

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[5] Abbreviations used: IL-1ra, IL-1 receptor antagonist; NK, natural killer; RCT, randomized controlled trial; RSV, respiratory syncytial virus; URTI, upper respiratory tract infection.
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had ≥1 URTI episodes in a 2-mo training period before the Los Angeles marathon. In New Zealand, a study reported >50% of a professional Super 15 Rugby team experienced URTI symptoms during a 4-wk training period (14). Consequently, researchers are looking for new and novel ways to decrease URTI incidence, one of which is flavonoids.

Flavonoids are "the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants" (15). Flavonoids are found in many different species of plants and can be subdivided into flavonols, flavones, flavanols, flavanones, anthocyanidins, proanthocyanidins, and isoflavones, each with their own individual compounds (16, 17). The widespread distribution of flavonoids and their tolerability mean that humans ingest appreciable quantities in their diet.

Flavonoids are proposed to reduce the incidence of URTIs because they have a range of physiologic effects in humans, including antiviral, anti-inflammatory, cytotoxic, antimicrobial, and antioxidant (18). Studies report flavonoids have both an antiproliferative and antireplicative effect on 2 common viral sources of URTIs and reduce inflammation by decreasing NF-κB (19–22). These mechanisms, and others, may have the potential to decrease URTI incidence, which makes flavonoids a current field of interest in human immunity (23–26).

Herbal and "natural" products are a growing industry in today's society because they reportedly help with numerous diseases and ailments (27). The purpose of this current systematic review is to investigate the efficacy of all food-related and supplementary forms of flavonoids on URTIs and immune function. Because athletes are reported to have an increased URTI risk, the effect of flavonoids on immune function after exercise is also investigated. In addition, analyzing the adverse effects assesses the applicability of a flavonoid supplement to society.

Methods

Search strategy and selection criteria. A search of all current literature that investigated the effect of flavonoids on URTIs was conducted by 2 reviewers (VSS and AJB) independently with the use of a created search strategy. The search was optimized for each database by using Medical Subject Headings, Boolean operators, and the Cochrane sensitive-maximizing randomized controlled trial (RCT) filter, when appropriate. The search strategy was run against Cochrane Central Register of Controlled Trials, MEDLINE, EMBASE and EMBASE classic, CINAHL, and AMED. The search strategy consisted of the following 3 main concepts: flavonoids, immune biomarkers, and URTIs. The meta-register of controlled trials, clinicaltrials.gov, the WHO International Clinical Trials Registry Platform, the System for Information on Gray Literature in Europe, and Google Scholar were also searched with the terms "Flavonoid and Immune."

Once all studies were identified, 2 reviewers (VSS and AJB) screened the titles and abstracts for potential inclusion and also deleted duplicates. Subsequently, 78 full-text articles remained, and their reference lists were reviewed for additional studies, but no further studies were identified. The 78 studies were evaluated against the inclusion and exclusion criteria independently by the 2 reviewers.

The inclusion criteria included the following:

1. Single- or double-blind RCT
2. Human participants aged between 18 and 65 y
3. Flavonoid intervention for ≥4 d
4. URTI incidence diagnosed by a medical professional or predetermined "illness log" as defined by the individual studies

Primary outcomes included incidence of URTIs and their respective duration and severity (days missed from work/school). Secondary outcomes included adverse effects and measures of immune status with the use of the following biomarkers: IL-6, IL-8, IL-10, IL-1 receptor antagonist (IL-1ra), TNF-α, IFN-γ, natural killer (NK) cell concentrations, neutrophil concentrations, and both $CD4^+$ and $CD8^+$ T-lymphocyte counts, and immune function after exercise (intervention compared with control).

The exclusion criteria were participants who smoked or had a history of, or current, cardiovascular/pulmonary/endocrinologic disease, malignancy, obesity, and/or immune defects or allergies (i.e., allergic rhinitis). Two authors (VSS and AJB) then came to a consensus as to which studies would be included. After this process, 14 studies remained for inclusion in the systematic review (**Figure 1**).

Data collection and assessment of bias. The review of abstracts and full texts retrieved followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (28). Collected data included characteristics of participants and study, intervention type and dose, study description/overview/setting, study recruitment, risk of bias, and outcomes. Unsuccessful attempts were made to contact 2 investigators for additional statistics (29, 30).

With the use of the following 6 categories, 2 reviewers also completed the assessment of the risk of bias for each study independently:

1. Sequence generation
2. Allocation concealment
3. Blinding
4. Missing outcome data
5. Risk of reporting bias
6. Other sources of bias according to the Cochrane Handbook (31) (**Figure 2**)

Data synthesis. For inclusion of a study estimate in the meta-analysis, investigators had to provide an SE, an exact *P* value, or other information that would allow calculation of the SE. URTI incidence and count of sick days were the only outcome measures reported with sufficient data for meta-analysis.

URTI incidence was adjusted for intervention duration by calculating the hazard (incidence per unit of time). The hazard in each group was assumed to be constant and given by hazard = $-\ln(1 - \text{incidence}/n)/t$, where t = duration of study. The HR was calculated by dividing the intervention hazard by the control hazard. The effect of the intervention on the count of sick days was also expressed as a ratio for meta-analysis. The ratio of sick days was calculated by dividing the intervention ratio (count of sick days/n) by the control ratio.

Data analysis. Meta-analyses were performed with the mixed model (Proc Mixed) in the Statistical Analysis System (version 9.4; SAS Institute) by using the method of holding residual variance to zero (32) or, when convergence failed, by using the method of holding variances of study estimates to their observed values (33).

The assumption was made that the *P* value provided by the investigators for their outcome could be applied to the ln of the HR and that the sampling distribution of the ratio was normally distributed. These assumptions allowed estimation of the SE of each estimate for the meta-analysis. The meta-analysis of count of sick days was performed with the ln of the ratio. The SE for the log of the ratio of sick days per participant in the intervention and control groups was derived by assuming the count of sick days had a Poisson distribution. Study estimates were weighted by the inverse of the squares of their SEs. Meta-analyzed outcomes were back transformed to HR or count ratios. All analyses included a random effect for true between-study variance, allowing for negative variance in SAS. We back transformed the square root of the variance and its CIs to create a real (free of within-study variation) between-study SD.

The remaining outcomes with insufficient data for meta-analysis are displayed in a log scatter plot (bio-immune markers) or described (count of symptoms, days missed, and adverse effects). For change in bio-immune markers over

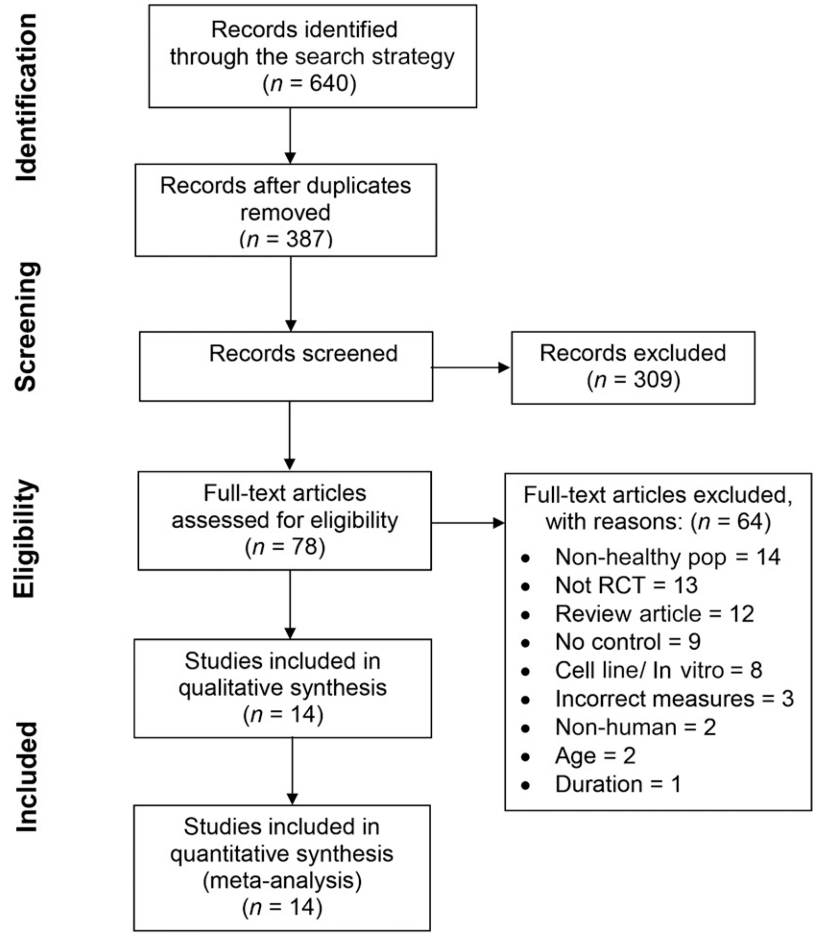

FIGURE 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart. Pop, population; RCT, randomized controlled trial.

time, the ratio (day measure/baseline measure) of intervention and control were both plotted against time. For change in bio-immune markers before compared with after exercise, the ratios (postexercise measure/pre-exercise measure) of intervention and control were both plotted in bio-immune marker columns.

Uncertainty of the effect estimates are reported as 95% CIs. In the analysis, magnitude-based inferences were made about the true value of the effect (34). In the interpretation of the HR, the outcome was deemed unclear if there was a possibility of benefit ($P > 0.25$) but unacceptable risk of harm ($P > 0.005$). If the result was deemed clear, thresholds were used to interpret the magnitude of the effect. The factor thresholds used for HR were 0.9, 0.7, and 0.5 for small, moderate, and large reductions, respectively, and inverse for increases (34). To interpret the magnitude of the bio-immune marker data, the baseline variability of the bio-immune marker was calculated as a ×/÷ factor SD. The factor SD was calculated by expressing each study SD as a factor [(SD + mean)/mean], log transforming, squaring, calculating the mean weighted by the degrees of freedom for the SD, then back transforming the outcome (Equation *1*). To assess the magnitude of change, thresholds of 0.20, 0.60, and 1.20 of the log-transformed factor SD were used, which represent small, moderate, and large change, respectively (34).

$$e^{\sqrt{\frac{\sum(\{\ln[(\text{mean}+\text{SD})/\text{mean}]\}^2 \cdot df)}{\sum df}}} \qquad (1)$$

Results

Study selection

After individual screening of the 387 articles and deletion of articles that did not fit the research according to title/abstract, then mutual consensus decision by the 2 reviewers of the remaining 78 articles, 14 studies were included for review. The selection process, including the number of studies at each stage and reasons for exclusion, are presented in Figure 1.

Study characteristics

Some of the extracted data from the studies are shown in Table 1. In summary, all of the studies were RCTs, 3 used a crossover design (38, 39, 47) and 11 used a parallel design (30, 35–37, 40–46). The studies were conducted in a range of countries, including 9 in the United States (36, 38–43, 45, 46), 3 in Germany (37, 44, 47), 1 in the United Kingdom (35), and 1 in Brazil (30). A total of 4 studies used individual flavonoid supplements, either anthocyanidins (35) or quercetin (36, 42, 43), whereas the remaining used a combination of flavonoids (30, 37–41, 44–47). In 5 trials, the specific flavonoid and/or amount were not reported (30, 37, 40, 44, 45). Of the remaining 9 trials that mentioned the dose, 5 used an intervention dose > 350 mg/d (35, 36, 41–43), which is above the average daily intake of flavonoids (48–53). The total dose ranged from 0.2 to 1.2 g of flavonoid daily.

The 14 studies can be subdivided into 3 durations. Bell et al. (35) and McAnulty et al. (39) had an intervention

TABLE 1 Summary of included studies on association between dietary flavonoids and URTIs and associated immune markers in healthy adults[1]

Study (ref)	Participants	Study design	Intervention	Outcomes measured
Bell et al., 2014 (35)	16 Well-trained male cyclists from the United Kingdom	Double-blind parallel RCT, 4-d supplement loading then exercise trial on days 5, 6, and 7	1.1 g anthocyanins/d for 7 d	Bio-immune markers (IL-6, IL-8, and TNF-α)
Henson et al., 2008 (36)	39 Adults from the United States completing the WSER	Double-blind parallel RCT, with 21-d supplementation before the WSER	1 g quercetin/d for 21 d	URTI (incidence and sick days) and bio-immune markers (NK cells and neutrophils)
Huber et al., 2011 (37)	41 Adults from Germany	Double-blind parallel RCT	Iscucin Populi (0.0125%, 0.25%, 5%), and Viscum Mali e planta tota (1:1000, 1:100, 2%) for 28 d	Bio-immune markers (IL-6, TNF-α, NK cell, neutrophils, $CD4^+$ and $CD8^+$ cells) and adverse effects
Knab et al., 2014 (38)	9 Elite male swimmers at SwimMAC Carolina	Single-blind crossover RCT	230 mg flavonoids/d by juice for 10 d, 21-d washout	Bio-immune markers (IL-6 and IL-10)
Luna et al., 2011 (30)	14 Adult men running the Sao Paulo marathon	Double-blind parallel RCT, 28-d supplementation before the marathon	72 g/d of (unquantifiable) intervention for 28 d	Bio-immune markers (IL-6, IL-8, IL-10, and TNF-α) and adverse effects
McAnulty et al., 2004 (39)	9 Moderately trained men of the Appalachian University area	Double-blind crossover RCT	150 g blueberries/d = 142 mg anthocyanins,[2] 7-d washout	Bio-immune markers (IL-6, IL-8, IL-10, IL-1ra, and TNF-α)
Nantz et al., 2012 (40)	112 Men and women from the University of Florida campus and Gainesville	Double-blind parallel RCT	2.56 g aged-garlic extract/d for 90 d	URTI (incidence, sick days, days missed at work, and total symptoms), bio-immune markers (TNF-α and IFN-γ), and adverse effects
Nantz et al., 2013 (41)	45 Men and women from the University of Florida campus and Gainesville	Double-blind parallel RCT	292.5–346.5 mL proanthocyanidins/d 30.6–50.85 mL anthocyanins/d 30.6–45 mL flavonols/d for 70 d	URTI (incidence, sick days, days missed at work or school, and total symptoms), bio-immune markers (TNF-α and IFN-γ), and adverse effects
Nieman et al., 2007a (42)	40 Trained male cyclists in North Carolina	Double-blind parallel RCT, supplementation 21 d before exercise, during 3-d period of intensified exercise, and 14 d after	1 g quercetin/d for 38 d	URTI (incidence and sick days) and bio-immune markers (NK cells)
Nieman et al., 2007b (43)	40 Trained male cyclists in North Carolina	Double-blind parallel RCT, supplementation 21 d before exercise, during 3-d period of exercise, and 14 d after	1 g quercetin/d for 38 d	Bio-immune markers (IL-6, IL-8, IL-10, IL-1ra, and TNF-α)
Riede et al., 2013 (44)	187 Men and woman from Germany	Double-blind parallel RCT	4.5 g proprietary water-based extract from larch tree (RestAid, contains taxifolin and quercetin)/d for 84 d	URTI (incidence) and adverse effects
Rowe et al., 2007 (45)	108 Men and women from University of Florida or the Florida community	Double-blind parallel RCT	Unknown quantity of L-theanine and epigallocatechin gallate for 84 d	URTI (incidence and sick days) and adverse effects
Ryan-Borchers et al., 2006 (46)	52 Postmenopausal women from the United States	Double-blind parallel RCT	Soymilk + control = 30.9 ± 1.5 mg diadzein/d, 37.4 ± 1.3 mg genistein/d, and 3.6 ± 0.5 mg glycitein/d = 71.6 ± 3.1 mg/d for 112 d Cow's milk + isoflavones = 30 mg diadzein/d, 33 mg genistein/d, and 7 mg glycitein/d = 70 mg/d for 112 d	Bio-immune markers (TNF-α, IFN-γ, and NK cells)
Schwarz et al., 2002 (47)	15 Men from the United States	Double-blind crossover RCT	*Echinacea purpurea* herb harvested without roots and containing 22% (vol:vol) ethanol for 14 d, washout 28 d	Bio-immune markers (TNF-α) and adverse effects

[1] IL-1ra, IL-1 receptor antagonist; NK, natural killer; RCT, randomized controlled trial; ref, reference; URTI, upper respiratory tract infection; WSER, Western States Endurance Run.
[2] Flavonoid content estimated from the USDA Flavonoid database.

TABLE 2 Pooled study characteristics and meta-analysis (SAS) of studies that investigated URTI incidence, displayed as an HR, whereby the hazard is the occurrence of ≥1 URTI episode in the study duration[1]

	Intervention		Control				
Study (ref)	Incidence	*n*	Incidence	*n*	Duration, d	*P*-value	HR[2]
Henson et al., 2008 (36)	4	18	5	21	14	0.88	0.92
Nantz et al., 2013 (41)	15	22	20	23	70	0.62	0.56
Nantz et al., 2012 (40)	26	56	28	56	90	0.85	0.9
Nieman et al., 2007a (42)	1	20	9	20	14	<0.01	0.09
Riede et al., 2013 (44)	58	97	67	90	84	0.03	0.67
Rowe et al., 2007 (45)	23	53	35	55	84	0.04	0.56

[1] ref, reference; SAS, Statistical Analysis System; URTI, upper respiratory tract infection.
[2] Overall effect: HR = 0.67 (95% CI: 0.64, 0.69).

duration of 1 wk; 6 studies had a duration of 1–4 wk (30, 36, 38, 42, 43, 47), and 6 studies had an intervention duration of 10–13 wk (37, 40, 41, 44–46).

The studies varied in their reported outcomes, although they all measured ≥1 of the predetermined primary or secondary outcomes, including 7 studies that reported adverse effects (30, 37, 40, 41, 44, 45, 47). Of the 14 studies included, 6 studies measured URTI incidence (36, 40–42, 44, 45); 4 measured sick days (36, 40, 42, 45); and only Nantz et al. (40, 41) measured days missed and URTI symptom count.

Bias

In summary, 6 studies reported adequate sequence of allocating participants to treatment (37, 40, 41, 44, 46, 47), whereas the remaining 8 did not state the method of allocation and therefore had unclear bias (30, 35, 36, 38, 39, 42, 43, 45) (**Figure 2**).

Similarly, 6 studies reported a satisfactory method of concealing allocation (35, 39–41, 45, 46), and the remaining 8 studies were categorized as unclear because the method was not reported by the investigators (30, 36–38, 42, 43, 45, 46).

All studies were double-blind RCTs except Knab et al. (38), which was a single-blind RCT. As a consequence, the 13 double-blind RCTs had a low blinding bias, and Knab et al. (38) had high blinding bias.

Most studies had zero withdrawals during the study period (30, 35, 37–41, 44, 46, 47); therefore, no outcome data were missing. Henson et al. (36) and Rowe et al. (45) provided differing allocation and follow-up numbers, but the reviewers were unable to confirm if the withdrawals were from the intervention or control group; consequently, these studies were allocated as unclear. Another 2 studies (42, 43) reported the total number of participants allocated rather than number of participants allocated to intervention and control, so it was not possible to derive dropout rates; subsequently, the studies were categorized as unclear.

Once the studies were included in the systematic review, a search for their associated protocol was conducted to assess the risk of reporting bias in each study. In 13 of the 14 studies, no protocol was found. These 13 studies were subsequently assigned as unclear bias. The protocol for Riede et al. (44) was found; however, one of the secondary outcomes mentioned in this protocol was not presented in the final study, so it was subsequently classified as high bias.

The main source of "other bias" resulted from funding. In 6 studies there was no concern about funding sources (30, 36, 40, 42, 43, 46). Dole Food Company, who had a vested interest, funded the study by Knab et al. (38). Although the investigators produced negative results, the study was still published. McAnulty et al. (39) was sponsored by the North American Blueberry Council; however, it is not a direct wholesaler of blueberries. Because it was not possible to estimate the impact funding had on these 2 studies, they were both allocated unclear. The remaining 6 studies were assigned high bias for various reasons; 4 studies had funding provided from the intervention supplement company (35, 37, 41, 45), Schwarz et al. (47) was funded by the investigators, and in Riede et al. (44), the funding company made the final decision about publication.

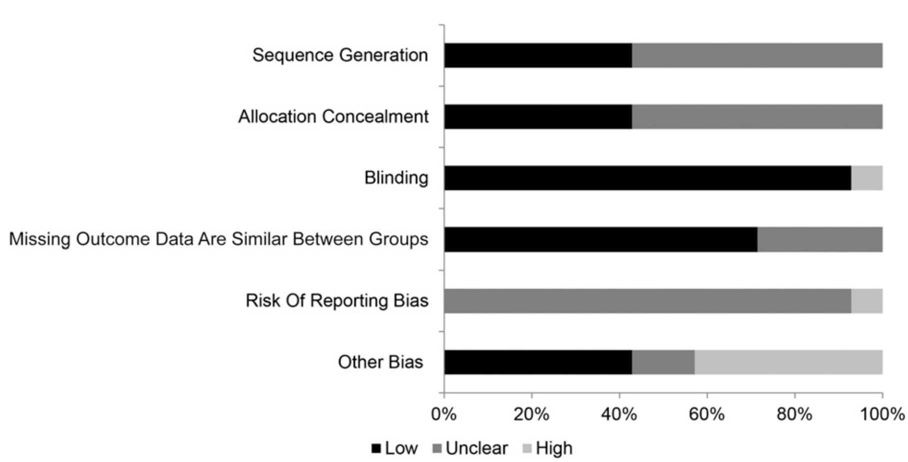

FIGURE 2 Risk of bias summary of the included studies in the systematic review.

URTI incidence

Six RCTs reported URTI incidence, totaling 531 participants (36, 40–42, 44, 45). All participants were given either a flavonoid intervention (n = 266) or control (n = 265). The meta-analysis of URTI incidence displayed as an HR, in which the hazard is ≥1 URTI episode in the study duration, is shown in Table 2. A clear moderate reduction was seen in participants who experienced ≥1 URTI on flavonoid supplementation compared with control. The real between-study variance reported by SAS was negative and expressed as a factor was 0.83 (95% CI: 0.75, 1.11). No meta-regression that controlled for any of our subanalysis categories was completed, because data were insufficient.

URTI sick days

Four studies reported sick days as a result of URTIs with intervention (n = 147) or control (n = 152) over a cumulative period of 202 d (36, 40, 42, 45). The meta-analysis of the URTI sick days is shown in (**Table 3**). Flavonoid supplementation had an unclear reduction in URTI sick days (P = 0.16). The real difference between studies, expressed as a factor, was 1.63 (95% CI: 0.62, 2.32). Data were insufficient to perform a meta-regression.

URTI severity

Days missed. Days missed from work/school give an estimate of URTI severity. Nantz et al. (40) reported a statistically significant reduction in work days missed in the flavonoid supplementation cohort compared with the control cohort (P = 0.035). Nantz et al. (41) described a similar reduction in work days missed; however, the result was not significant (P = 0.33).

Symptoms. The severity of URTIs can also be represented by the number of URTI symptoms of that episode. Only Nantz et al. (40, 41) investigated the number of symptoms due to URTI episodes. Both studies reported statistically significant reductions in URTI symptoms with flavonoid intervention compared with control intervention (P < 0.001 and P = 0.031, respectively).

TABLE 3 Study characteristics and meta-analysis (SAS) of studies that investigated URTI sick-day count, displayed as a sick-day ratio (intervention sick-day count/control sick-day count), whereby the sick-day count is the number of sick-days divided by the number of participants[1]

	Intervention		Control		Duration,		Sick-day
Study (ref)	Incidence	*n*	Incidence	*n*	d	*P*-value	ratio[2]
Henson et al., 2008 (36)	21	18	29	21	14	0.84	0.84
Nantz et al., 2012 (40)	317	56	358	56	90	0.13	0.89
Nieman et al., 2007a (42)	8	20	61	20	14	<0.01	0.13
Rowe et al., 2007 (45)	360	53	559	55	84	0.02	0.67

[1] ref, reference; SAS, Statistical Analysis System; URTI, upper respiratory tract infection.
[2] Overall effect: sick-day ratio = 0.60 (95% CI: 0.24, 1.47).

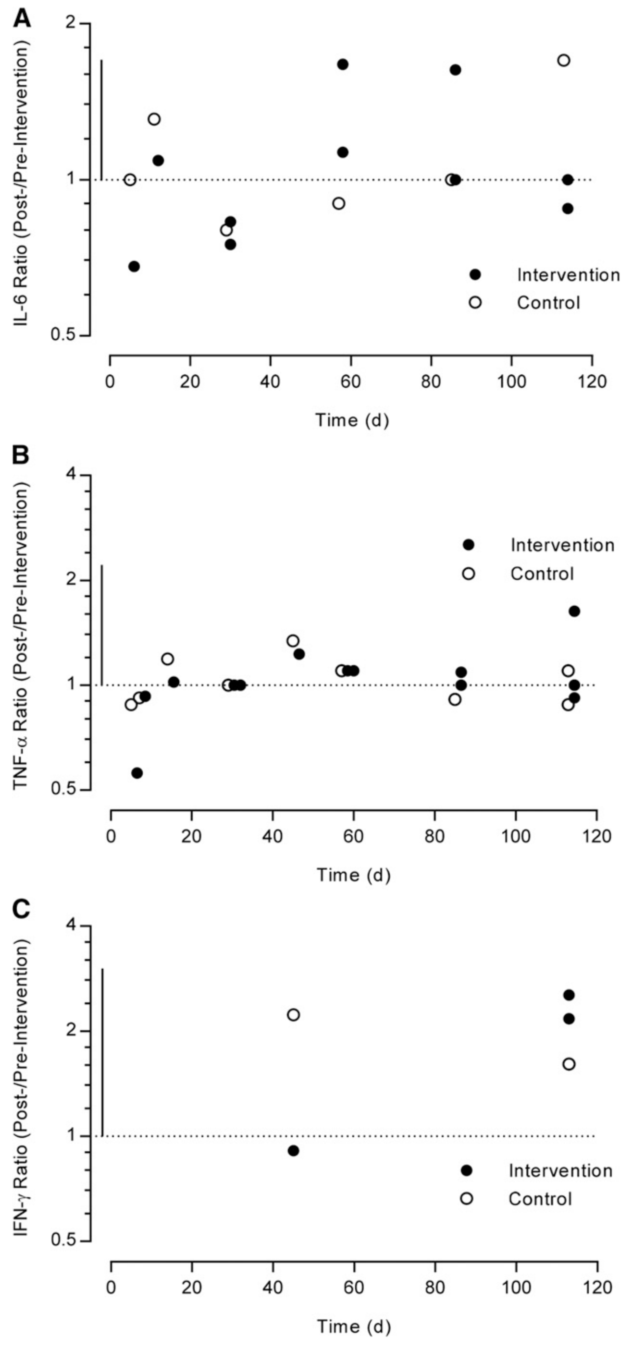

FIGURE 3 Log scale scatter plot of the studies that investigated cytokine concentration during their intervention period compared with baseline, expressed as a ratio (after:before intervention). The horizontal dotted line represents no change compared with baseline, and the vertical solid line represents 1-factor SD. (A) IL-6, (B) TNF-α, and (C) IFN-γ ratios (after:before intervention or control) in healthy adults at different time points.

Effects of intervention on secondary outcomes

Bio-immune markers. The bio-immune markers were unable to be meta-analyzed and are therefore displayed in log scatter plots.

The intervention group reported small-to-moderate changes in IL-6 and IFN-γ and trivial-to-small changes

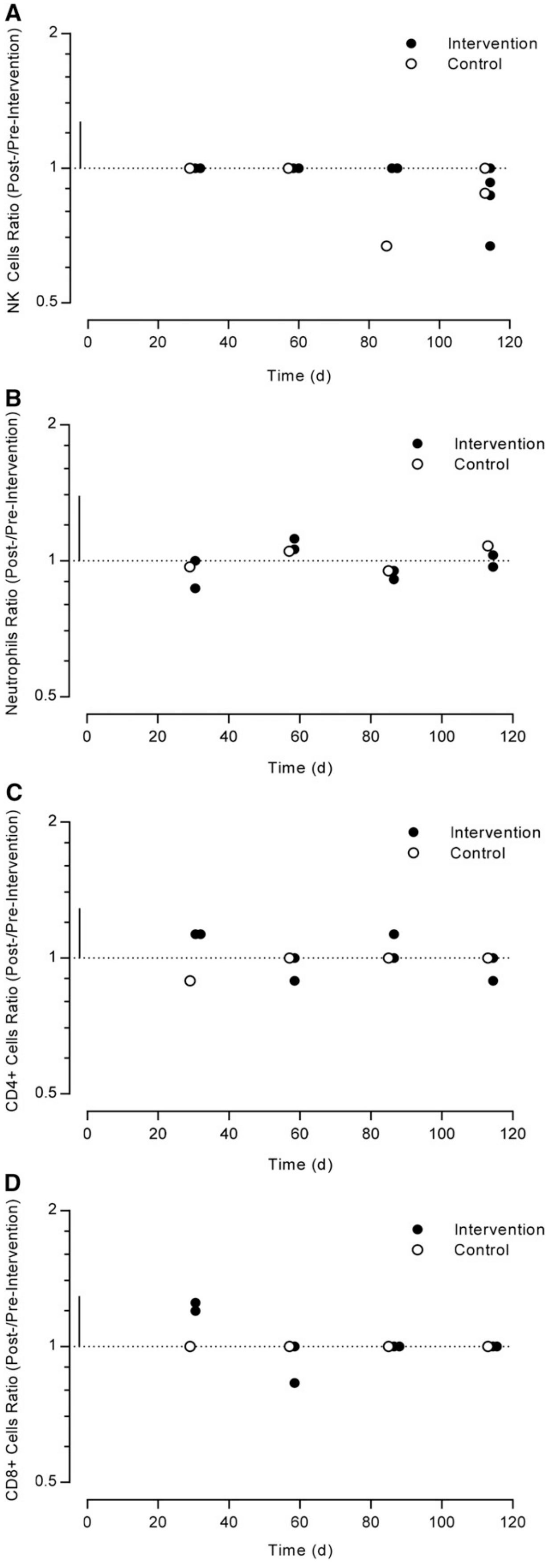

FIGURE 4 Log scale scatter plot of the studies that investigated cell concentration during their intervention period compared with baseline, expressed as a ratio (after:before intervention or control). The horizontal dotted line represents no change compared with baseline, and the vertical solid line represents 1-factor SD. (A) NK cell, (B) neutrophil, (C) $CD4^+$, and (D) $CD8^+$ ratios in healthy adults at different time points. NK, natural killer.

in TNF-α (**Figure 3**). The magnitudes of the change in the intervention group, however, were similar in size to the control group. Data on IL-8 and IL-10 are not presented because they are only reported by Bell et al. (35) and Knab et al. (38), respectively. No studies reported IL-1ra measures through the trial duration.

All cell concentrations (NK, neutrophil, $CD4^+$, and $CD8^+$) similarly had a range of small, moderate, and large changes with flavonoid intervention over time (**Figure 4**). The intervention changes were, again, of similar magnitude to the control changes.

All cell and cytokine measures were raised after exercise, except NK cells, which decreased (**Figure 5**). The magnitudes of the change in the geometric means of the intervention measures were varied with extremely large (IL-6, IL-10, IL-1ra, and neutrophils), moderate (IL-8), and small (TNF-α and NK cell) changes. Although, according to the nonexercise data, the magnitude change in the intervention group was similar in the control group. No studies reported data before compared with after exercise for either $CD4^+$ or $CD8^+$ cells.

Adverse effects. Seven studies reported specific numbers of, or commented on, adverse effects (30, 37, 40, 41, 44, 45, 47). Four studies reported no adverse effects (30, 40, 41, 47), 2 reported equal numbers of adverse effects (44, 45), and one arm of the Huber et al. (37) study reported 18 side effects in the intervention group compared with 7 in the control group. The Huber et al. (37) arm that reported 18 adverse effects was an anticancer treatment and consequently contained cytotoxic lectins.

Discussion

To our knowledge, this is the first meta-analysis and systematic review conducted to examine the effect of flavonoids on URTIs and associated bio-immune markers in humans. The primary outcomes of this study are that flavonoids decrease URTI incidence by 33% compared with control with no increase in adverse effects and that a nonsignificant decrease was found in URTI severity and duration with the flavonoid intervention. Furthermore, trivial differences were found in the secondary outcomes (bio-immune markers throughout the duration of the intervention and before/after exercise) for participants in the intervention group compared with the control group. In addition, Nantz et al. (40, 41), the only studies to measure symptoms and severity of URTIs, reported statistically significant reductions in URTI symptoms

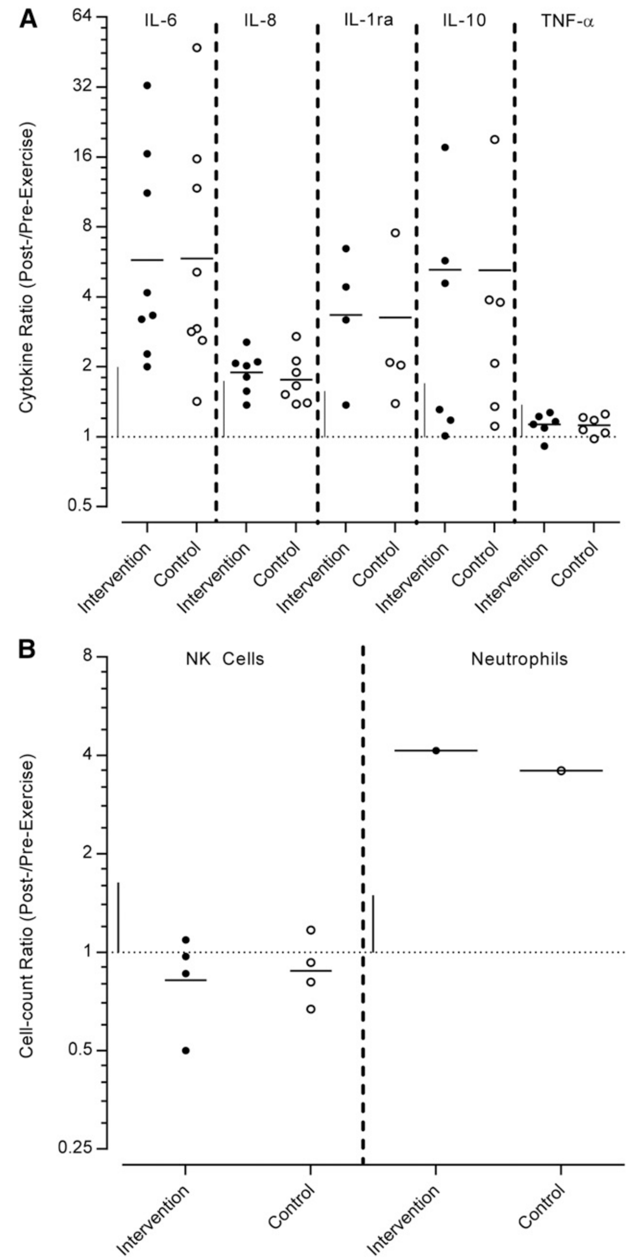

FIGURE 5 Log scale scatter plot of the studies that investigated cytokine and cell concentrations before and after exercise, after a specified intervention duration, expressed as an after:before exercise ratio. The horizontal dotted line represents no change compared with baseline. The horizontal solid line represents the geometric mean, and the vertical solid line represents 1-factor SD. (A) Cytokine and (B) cell ratios of measures, defined by each study in a range of healthy trained or exercising adults. NK, natural killer.

with the flavonoid intervention and also reported a statistically significant reduction in days missed due to URTIs (40).

Six RCTs (n = 531) were identified that investigated the effect of flavonoids on URTI incidence. Participants taking the flavonoid intervention had a decreased URTI incidence compared with the control intervention, which is in agreement with theoretical evidence. Several secondary outcomes in this systematic review that could potentially explain the reduction of URTI incidence were also investigated. Previous research has suggested that flavonoids have an antiviral, anti-inflammatory, cytotoxic, antimicrobial, and antioxidant capacity to benefit the immune system (18). The current meta-analysis demonstrated only trivial differences between flavonoids and control for all immune biomarkers which suggests flavonoids do not attenuate URTI incidence by altering cytokine and immune cells in a beneficial way to the host. Because URTIs are primarily of viral origin, antimicrobial action will be of no potential benefit. From the data collected in this systematic review, it leads the researchers to propose that flavonoids decrease URTI incidence and symptoms by an antiviral mechanism. Currently, both observational and experimental data support flavonoids having antiviral activity (41). Kaul et al. (19) showed that particular flavonoids have different effects on viruses; quercetin and hesperetin have an antireplicative effect on RSV and parainfluenza virus type 3, whereas catechin and quercetin have an anti-infective effect on RSV. Quercetin was observed to have a dose-response effect, whereas naringin had no effect on the viruses (19).

When analyzing the immune function after exercise, all cytokines and cells increased or decreased with similar magnitude with or without flavonoid intervention. This suggests that flavonoids have no effect in moderating the change in bio-immune markers after exercise. Nieman et al. (54) reported that the "open window," during which athletes had increased susceptibility, was 3–72 h after exercise. Given that all these studies reported measures immediately after exercise and not up to 72 h after exercise, this review is unable to deduce if flavonoids have an effect on the bio-immune markers in the open window.

Application

Further research is needed to quantify the optimal dose of flavonoids. Two clear moderators of the effect of flavonoids on URTI incidence are the dose and type, which also contribute to the practical application of a flavonoid intervention. We were only able to quantify the dose and/or type of flavonoid intervention in 3 trials included in this meta-analysis (36, 41, 42). As a result, data were insufficient to perform a subgroup analysis. This information is meaningful because it describes the optimal prescription of flavonoid intake to achieve the decrease in URTI incidence. Of the 3 studies that did provide the quantity of flavonoid, the intervention dose was greater than the average daily intake. It is therefore plausible that average daily intake of flavonoids is insufficient to reduce URTI incidence and that an increased intake is needed. Intake of flavonoids (~1000 mg) can be achieved by diet through simple practices such as consuming green tea (250 mL), a glass of Shiraz (250 mL), blueberries (100 g), or some dark chocolate (100 g), which are all products high in flavonoid content (55). Similarly, the increased flavonoid intake could also be provided by a flavonoid supplement. The method of flavonoid increase will depend on which is of greater ease and accessibility to the general public.

This meta-analysis has quantified variation, and, as a result, a researcher can expect this magnitude of variation in the mean effect, irrespective of setting. This variance can be attributed to a few mechanisms, such as inadequate reporting of error and measures, unknown cofactors that also influence the incidence of URTIs and symptoms, and a meta-analytic model that fails to incorporate the full nature of reality, including nonlinear effects and interactions. In URTI incidence, the between-study variation interval means that researchers or practitioners are likely to see a benefit of flavonoid supplementation despite their differing settings. Conversely, the large interval of sick days suggests that researchers may not see a reduction in URTI sick days in their respective setting.

Finally, no increases in adverse effects were reported in the studies, except in 1 arm of the Huber et al. (37) study in which the intervention contained a cytotoxic lectin component. This is important when delivering a food-based health claim that could be used by the public. In this systematic review no notable increase was found in adverse effects; therefore, members of society could take flavonoid supplements with reasonable confidence that the flavonoid component will not result in increased adverse events.

Conclusion

The research presented in this systematic review demonstrates the essential role of flavonoids in the function of the respiratory immune system. To our knowledge, this is the first systematic review and meta-analysis to assess the effects of flavonoids on URTI incidence, severity and duration, bio-immune markers, and adverse effects. The findings demonstrate that flavonoids decrease URTI incidence compared with control with no increase in adverse effects. Although flavonoid intervention decreases URTI incidence, no conclusive evidence proves that flavonoids decrease duration or severity. We also identified that bio-immune marker measures have a similar magnitude of change over the intervention duration and after exercise in the intervention and control groups.

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