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The effect of nut consumption on markers of inflammation and endothelial function: a systematic review and meta-analysis

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Abstract

Objectives: To examine the effect of nut consumption on inflammatory biomarkers and endothelial function.

Design: A systematic review and meta-analysis

Data sources: Medline, PubMed, CINAHL and Cochrane Central Register of Controlled Trials (all years to 13 January 2016)

Eligibility criteria: Randomised controlled trials (with a duration of three weeks or more) or prospective cohort designs conducted in adults; studies assessing the effect of consumption of tree nuts or peanuts on C-reactive protein (CRP), adiponectin, tumour necrosis factor-alpha, interleukin-6, intercellular adhesion molecule 1, vascular cell adhesion protein 1, and flow mediated dilation (FMD).

Data extraction and analysis: Relevant data was extracted for summary tables and analyses by two independent researchers. Random effects meta-analyses were conducted to explore weighted mean differences (WMD) in change or final mean values for each outcome.

Results: A total of n=32 studies were included in the review. Consumption of nuts resulted in significant improvements in FMD (WMD: 0.79 [0.35, 1.23]). Non-significant changes in biomarkers of inflammation were found, although sensitivity analyses suggest results for CRP may have been influenced by two individual studies.

Conclusions: This systematic review and meta-analysis of the effects of nut consumption on inflammation and endothelial function found evidence for favourable effects on FMD, a measure of endothelial function. Non-significant changes in other biomarkers indicate a lack of consistent

evidence for effects of nut consumption on inflammation. The findings of this analysis suggest a need for more research in this area, with a particular focus on randomised controlled trials

Review registration: CRD42016045424

Strengths and limitations of this study

- This is the first known systematic review and meta-analysis which examined the effect of nut consumption on inflammation and endothelial function, in studies which isolated the effect of nut consumption
- The protocol for the review was pre-registered, and the review followed the requirements of the PRISMA statement
- Risk of bias was assessed using the Cochrane Risk of Bias Tool, and the quality of the body of evidence was then determined using GRADE
- The available evidence base for some of the biomarkers explored was small
- There were variations in the included studies, such as participant health status, nut type and dose, and study duration, although these factors were explored in sub-group analyses

INTRODUCTION

Chronic conditions such as type 2 diabetes, and metabolic syndrome are known to be underpinned by a state of low-grade inflammation, which play a central role in disease progression, and in the development of atherosclerosis[1 2]. Changes in this inflammatory state can be identified via biomarkers of inflammation including C-reactive protein (CRP)[3], tumour necrosis factor-alpha (TNF-α)[4], interleukin-6 (IL-6)[5], and the adhesion molecules intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion protein 1 (VCAM-1)[6], as well as anti-inflammatory biomarkers such as the adipocyte adiponectin[7]. Endothelial dysfunction is a central component in the development and progression of atherosclerosis, with brachial flow mediated dilation (FMD), a non-invasive measure of endothelial function, found to be significantly associated with risk of cardiovascular events[8].

Given that markers of inflammation and endothelial function can indicate changes in disease development and progression, they can be used to explore the impact of consumption of specific foods on health. Nuts contain a wide range of nutrients and bioactive components which may moderate inflammation and the development of endothelial dysfunction, such as alpha-linolenic acid, L-arginine, fibre, and polyphenols[9]. Habitual nut intake has been associated with reduced risk of cardiovascular disease[10], decreased incidence of the metabolic syndrome[11], and decreased risk of diabetes[12]. Clinical trials have previously explored the effects of nut consumption on markers of inflammation and endothelial function, with a range of effects observed[13-22]. A systematic review and meta-analysis would consolidate and appraise the quality of this body of evidence, providing greater clarity where inconsistencies are observed. Even so, the effort is ongoing. For example, a recently published systematic review did not report significant effects of nut consumption on CRP[23], but did not include results of the large PREDIMED study[24]. It is

also possible to consider FMD as an outcome which this previous review did not consider. The aim of the review reported here was to examine the effect of nut consumption on inflammatory biomarkers and endothelial function in adults. It was hypothesized that the regular inclusion of nuts in a diet would improve markers of inflammation and endothelial function.

METHODS

This systematic review and meta-analysis followed the requirements of the PRISMA statement[25] (Supplementary material 1). The review was registered in PROSPERO, the international prospective register of systematic reviews (http://www.crd.york.ac.uk/PROSPERO; registration number: CRD42016045424).

Study selection

A systematic search of the databases Medline, PubMed, CINAHL and Cochrane Central Register of Controlled Trials was conducted (all years to 13 January 2016). Where possible, Medical Subject Heading (MeSH) terms as well as free-text search terms were used in the search, in line with current recommendations[26]. Reference lists of eligible articles and relevant reviews were also reviewed for potential studies. An example of the search strategy used is shown in Supplementary material 2. Articles were restricted to those published in English.

To be included in this review, studies were required to meet the following inclusion criteria: 1) randomised controlled trial (including both parallel and cross-over designs) or prospective cohort design; 2) studies conducted in humans aged 18 years or older; 3) studies assessing the effect of consumption of tree nuts or peanuts on an outcome of interest (CRP, adiponectin, TNF-alpha,

IL-6, ICAM-1 VCAM-1, FMD), where the effect of nut consumption could be isolated; 4) studies with an intervention duration of three weeks or more (in the case of randomised controlled trials). In addition, the following exclusion criteria were applied: 1) studies involving pregnant or breastfeeding women; 2) studies exploring the effects of nut oils or extracts.

Articles were screened based on title and abstract. Full texts were retrieved in the case that an abstract was not available or did not provide sufficient information to draw a conclusion regarding inclusion in the current review. In the case that results from one study were reported in multiple articles, data from only one article per outcome was extracted to avoid duplication of study populations in the analysis. Where there were multiple articles from one study, decisions relating to article inclusion were based first on the length of follow-up for the outcome, and then by sample size.

Data extraction

The following data were extracted from each study: citation, country, sample size, participant age and body mass index, health status, study design, study duration, nut type, nut dose, details of control arm, and background diet. Mean changes in relevant outcomes were extracted where possible, and in the case that this data was not available, mean final values were retrieved as recommended by the Cochrane Handbook for Systematic Reviews of Interventions[27]. Study authors were contacted for additional details if the published article did not provide sufficient information. Where a study involved more than one intervention group meeting the inclusion criteria, data for the two intervention groups were combined as recommended by the Cochrane Handbook[27]. In the case of the PREDIMED study[24], which included two intervention arms featuring a Mediterranean diet supplemented with either nuts or olive oil, and a low fat control

arm, data from the arm receiving the Mediterranean diet with olive oil was treated as the comparator group. This decision was made to ensure outcomes were not confounded by differences in the background diet of the two groups. Where studies reported median rather than mean, standard deviation was imputed from interquartile range.

Abstract screening, study inclusion and exclusion, and data extraction were conducted independently by two authors (EN and VG), and any disagreements were resolved via consensus.

Statistical analyses

Review Manager (Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, the Cochrane Collaboration, 2014) was used to conduct random effects meta-analyses to determine the weighted mean differences (WMD) (with 95% confidence intervals) in change or final mean values for each outcome. In initial analyses, cross-over studies were treated in the same way as parallel studies, as the most conservative approach to managing cross-over studies[27]. In order to explore whether this approach affected the final result by under-weighting these studies, paired analyses of cross-over studies using correlation coefficients of 0.25, 0.5, and 0.75 were conducted as sensitivity analyses.

Chi-squared tests were used to explore the consistency of the weighted mean differences for each outcome. I^2 was calculated based on the formula: $I^2 = 100\% \times (Q - df)/Q$ (where Q refers to the chi-squared statistic, and df refers to the degrees of freedom)[28]. An I^2 value of 75% or greater was deemed to indicate a high level of inconsistency, based on the recommendations by Higgins et al. [28]. For outcomes with ten or more strata, publication bias was explored using funnel plots, with Egger's test used to determine the extent of funnel plot asymmetry[29].

In addition to the correlation coefficient sensitivity analyses outlined previously, sensitivity analyses were also conducted to explore the effect of removing studies with imputed standard deviations from analyses, and of removing each individual study in meta-analyses ("leave-one-out" analysis). Pre-specified sub-group analyses were also conducted, based on study duration (less than three months versus more than three months), risk of bias, and nut type. For the purpose of sub-group analyses, studies which compared the effects of two types of nuts to a control[30 31] were classified as 'mixed nut studies'. Post-hoc sub-group analyses were conducted based on health status of participants, and whether the energy value of nuts was substituted for other foods.

Quality assessment

The Cochrane Collaboration Risk of Bias tool[27] was used to determine the risk of bias in included studies. EN and VG separately appraised the risk of bias and disagreements were resolved by discussion until consensus was reached. The quality of the body of evidence was then determined using GRADE[32]. GRADEproGDT software (GRADEpro. [Computer program on www.gradepro.org]. Version April 2015. McMaster University, 2014) was utilized to conduct the quality of evidence appraisal.

RESULTS

Characteristics of included studies

A total of n=5200 articles were identified from the systematic search and review of relevant reference lists. After applying exclusion criteria, n=36 articles describing n=32 studies (n=34

strata in pooled analyses) were included in the systematic review and meta-analysis. The process of study inclusion and exclusion is shown in Figure 1. Data access is available on request.

Characteristics of included studies are shown in Table 1. All included studies were randomised controlled trials. Fourteen studies had a parallel design[13 15 16 19 30 33-45], 17 had a cross-over design[14 17 18 20-22 31 46-55]. One study[56] combined a parallel and cross-over design, where participants were initially randomised to one of two parallel groups (energy adjusted or ad libitum diet). In this study, each group then took part in the cross-over part of the study consisting of a walnut included period and a walnut excluded period. Amongst all studies, duration ranged from four weeks to five years. Studies were conducted in Spain[16 18 20 31 33 38-42 48], the United States[14 17 22 34 36 43 45 47 49 50 53 54 56], Australia[44 46], India[19 35], Canada[51], South Korea[15], China[21], Brazil[37], South Africa[30], Iran[52], New Zealand[13], and Germany[55]. Studies included participants who were healthy[44 47], had risk factors for chronic disease such as overweight or obesity, dyslipidaemia, hypertension, or pre-diabetes[13 17 18 20 31 35-37 42 45 46 48 50 51 53-55], had type 2 diabetes mellitus[14 21 22 43 52], met the criteria for Metabolic Syndrome[15 16 19 30 33], had diagnosed coronary artery disease[49], or included a mixture of the aforementioned conditions[34 38-41 56]. Included studies examined the effects of consumption of a range of tree nuts including walnuts[17 18 22 34 45 47 48 50 55 56], almonds[21 36 43 49 51 53], pistachios[14 19 20 35 52 54], hazelnuts[13 42], mixed nuts[15 16 33 38-41], and Brazil nuts[44], as well as peanuts[37 46]. In addition, two studies included multiple intervention arms, featuring a different type of nut in each(walnuts and cashews[30], and walnuts and almonds[31]), compared to a control arm. Nuts were consumed in either prescribed doses, ranging from approximately 18[44] to 85 grams per day[49], or were designed to provide a set proportion of dietary energy, so the amount would vary for individuals[14 18 19 21 30 45 53 54]. Background diets consisted of either participant's habitual diet, which could be anything, or a

prescribed diet aligned with healthy lifestyles such as the NCEP Step I or II diet, a Mediterranean-style diet, the Therapeutic Lifestyle Changes diet or another prudent style diet in line with dietary guidelines. Six studies provided all or the majority of foods under controlled feeding conditions[14 21 30 50 53 54]. Twenty-two studies[14 17-22 30 31 34 35 37-42 45 48-51 53-55] prescribed diets accounting for the energy value of the nuts, either quantitatively through dietary modelling (including the energy value of the nuts within the total energy value of the diet) or qualitatively by encouraging participants to substitute nuts for items with similar energy values. One study[56] included an intervention group where participants were advised on food substitutions to account for the energy value of the provided nuts, and another intervention group where energy intake was not prescribed (ad libitum food consumption). During the control diets or periods, participants typically consumed a similar diet but without nuts, although some studies included control diets with a specific product substituted for the nuts, such as eggs[47], olive oil[31 38-41], muffins[51], and chocolate[36], amongst others. Only two studies[37 45] stated they prescribed a set energy restriction for both intervention and control groups; all other studies utilised isocaloric diets for weight maintenance or ad libitum diets. No studies reported a significant difference in weight loss between the intervention and control groups.

Table 1: Characteristics of included randomised controlled trials examining the effect of nut consumption on inflammatory biomarkers and endothelial function

Citation and country	Sample size (for analysis)	Mean age, years	Mean BMI, kg/m^2	Population	Design	Study duration, weeks	Nut type	Nut dose	Comparison group details	Background diet
Barbour et al. (2015)[46], Australia	61 (M: 29, F: 32)	65 ± 7	31 ± 4	Overweight	X	12	Peanut (high oleic)	M: 84g, 6 x week F: 56g, 6 x week	No nuts	Habitual diet
Burns-Whitmore et al. (2014)[47], United States	20 (M: 4, F: 16)	38 ± 3	23 ± 1	Healthy	X	8	Walnut	28.4g, 6 x week	Standard egg, 6x week*	Habitual diet
Canales et al. (2011)[48], Spain	22 (M: 12, F: 10)	54.8 (SEM: 2.0)	29.6 (SEM: 0.7)	Overweight with at least one risk factor for CVD	X	5	Walnut	150g/week walnut paste integrated into steaks and sausages	Low-fat steaks and sausages	Habitual diet with substituted meat products
Casas-Agustench et al. (2011)[16], Lopez-Uriarte et al. (2010)[33], Spain	50 (M: 28, F: 22)	*I:* 52.9 ± 8.4 *C:* 50.6 ± 8.4	*I:* 31.6 ± 2.8 *C:* 30.0 ± 3.3	MetS	P	12	Mixed nuts (walnut, almond, hazelnut)	30g/day (15g walnuts, 7.5g almonds, 7.5g hazelnuts)	No nuts	American Heart Association dietary guidelines
Chen et al. (2015)[49], United States	45 (M: 18, F: 27)	61.8 ± 8.6	30.2 ± 5.1	CAD	X	6	Almond	85g/day	No nuts	NCEP Step 1 diet (isocaloric)
Chiang et al. (2012)[50], United States	25 (M: 14, F: 11)	33 (range 23 - 65)	24.8 (range: 18.7 - 36.6)	Normal to HL	X	4	Walnut	42.5g per 10.1MJ (6 x week)	No nuts or fatty fish*	American Dietary Guidelines (isocaloric)
Damasceno et al. (2011)[31], Spain	18 (M: 9, F: 9)	56 ± 13	25.7 ± 2.3	HC	X	4	1.Walnut 2. Almond	1. 40 - 65g/day walnuts 2. 50 - 75g/day almonds	35 – 50g/day virgin olive oil	Mediterranean-style diet (isocaloric)
Djousse et al. (2016)[34], United States	26 (M: 10, F: 16)**	*I:* 60.8 ± 11.3 *C:* 68.8 ± 10.9	*I:* 29.6 ± 5.2 *C:* 33.5 ± 8.7	CAD or T2DM	P	12	Walnut	28g/day	No nuts	Habitual diet with walnuts substituted for equivalent kJ

										items
Gulati et al. (2014)[19], India	68 (M: 37, F: 31)	42.5 ± 8.2	30.9 ± 7.5	MetS	P	24	Pistachio	20% of total energy	Dietary guidelines for Asian Indians	Dietary guidelines for Asian Indians, with pistachios substituted for diet components
Hernandez-Alonso et al. (2014)[20], Spain	54 (M: 29, F: 25)	55 (95% CI: 53.4, 56.8)	28.9 (95% CI: 28.2, 29.6)	Pre-diabetic	X	16	Pistachio	57g/day	Intake of fatty foods adjusted to account for energy from pistachios	Isocaloric diet
Hu et al. (2016)[44], Australia	21 (M, F)‡‡	*I*: 62.4 ± 8.8 *C*: 66.5 ± 6.9	*I*: 82.2 ± 10.8 *C*: 83.9 ± 22.4§§	Healthy	P	6	Brazil nut (plus green tea extract)	18g/day¶¶	Green tea extract, no nuts	Habitual diet
Jenkins et al. (2002)[51], Canada	27 (M: 15, F: 12)	64 ± 9	25.7 ± 3.0	HL	X	4	Almond	73 ± 3 g/day¶¶	147 ± 6 g/day muffins¶¶,*	NCEP Step 2 diet (isocaloric)
Kasliwal et al. (2015)[35], India	56 (M: 46, F:10) (randomised) 42 (completed)	39.3 ± 8.1††	*I:* 26.1 ± 2.9†† *C:* 27.8 ± 4.7††	DL	P	12	Pistachio	40g/day shelled	No nuts	Therapeutic Lifestyle Change diet
Katz et al. (2012)[17], United States	46 (M: 18, F: 28)	57.4 ± 11.9	33.2 ± 4.4	Overweight plus risk factors for MetS	X	8	Walnut	56g/day	No nuts	Ad libitum, participants advised to substitute walnuts for other foods
Kurlansky and Stote (2006)[36], United States	47 (F)	*Almond:* 41.8 ± 11.7 *Almond + chocolate:* 46.2 ± 7.8 *Chocolate:* 36.5 ± 11.9 *C:* 51.3 ± 6.3	*Almond:* 25.3 ± 3.5 *Almond + chocolate:* 27.2 ± 4.2 *Chocolate:* 23.9 ± 3.3 *C:* 26.1 ± 4.1	Healthy, including HC	P	6	Almond	1. 60g/day 2. 60g almonds/ day + 41g dark chocolate/day	1. 41g dark chocolate/day 2. self-selected diet	Therapeutic Lifestyle Change diet (isocaloric)
Lee et al. (2014)[15], South Korea	60 (M, F)‡‡	ages 35 - 65 eligible for study	*I:* 27.19 ± 2.11 *C:* 26.96 ± 2.16	MetS	P	6	Mixed nuts (walnut, pine nut, peanut)	30g mixed nuts/day (15g walnuts, 7.5g pine nuts, 7.5g peanuts)	Prudent diet	Prudent diet (isocaloric)
Liu et al. (2013)[21], China	20 (M: 9, F: 11)	58 ± 2	26.0 ± 0.7	T2DM and HL	X	4	Almond	56g/day¶¶ (20% energy)	NCEP Step II diet	NCEP Step II diet (isocaloric diet)

Ma et al. (2010)[22], United States	24 (M: 10, F: 14)	58.1 ± 9.2	32.5 ± 5.0	T2DM	X	8	Walnut	56g/day	No nuts	Ad libitum, participants advised to substitute walnuts for other foods
Moreira Alves et al. (2014)[37], Brazil	65 (M)	*High oleic peanuts:* 27.2 ± 6.1 *Peanuts:* 27.6 ± 1.5 *C:* 27.1 ± 1.6	29.8 ± 2.3	Overweight	P	4	Peanut (high oleic and con-ventional)	1. 56g/day high oleic peanuts 2. 56g/day conventional peanuts	No peanuts	Hypocaloric diet (250 kcal/day deficit)
Mukuddem-Petersen et al. (2007)[30], South Africa	64 (M: 29, F: 35)	45 ± 10	*Walnut:* 36 (95% CI: 33.3 - 38.7) *Cashew:* 34.4 (95% CI: 32.3 - 36.6) *C:* 35.1 (95% CI: 32.8 - 37.4)	MetS	P	8	1. Walnut 2. Cashew	1. 20% energy from walnuts 2. 20% energy from cashews	No nuts	Controlled feeding protocol (isocaloric)
Njike et al. (2015)[56], United States	112 (M: 31, F: 81)	*Ad libitum:* 56.5 ± 11.7 *Energy adjusted:* 53.3 ± 11.1	*Ad libitum:* 30.0 ± 4.0: *Energy adjusted:* 30.2 ± 4.1	Overweight, pre-diabetic or MetS	X••	24	Walnut	56g/day	No nuts	1. Ad libitum diet 2. Isocaloric diet (energy adjusted for walnuts)
Parham et al. (2014)[52], Iran	44 (M: 11, F: 33)	*Intervention first:* 53 ± 10 *Control first:* 50 ± 11	*Intervention first:* 32.16 ± 6.58 *Control first:* 30.24 ± 4.03	T2DM	X	12	Pistachio	50g/day	No pistachios	Ad libitum
PREDIMED (Casas et al., 2014[38], Casas et al., 2016[39], Lasa et al., 2014[40], Urpi-Sarda et al., 2012[41]), Spain	353 (M: 172, F: 181)‡ 124 (M: 45, F: 79)• 110 (M: 55, F: 55)§ 108 (M: 54, F: 54)¶	Range: 55 – 80 (M), 60 – 80 (F)	29.4 ± 3.4‡	T2DM and/or CHD risk factors	P	52 ‡,•,§ 260 (5 years)¶	Mixed nuts (walnut, almond, hazelnut)	30g/day (15g walnuts, 7.5g hazelnuts, 7.5g almonds)	1L olive oil per week†	Mediterranean diet
Rajaram et al. (2010)[53], United States	25 (M: 14, F: 11)	41 (SEM: 13)	71 (SEM: 2.7)§§	Healthy (including overweight) to HC	X	4	Almond	1. 10% energy 2. 20% energy	No nuts	Cholesterol lowering diet (isocaloric)
Rock et al.	126 (F)	50 (range: 22 -	33.5 (range:	Overweight	P	52	Walnut	42g/day¶¶	1. higher fat	Hypocaloric diet

(2016)[45], United States		72)††	27 - 40)††					(18% energy)	(35% energy) lower CHO (45% energy) diet, no nuts*	(500 - 1000 kcal/day deficit)
Ros et al. (2004)[18], Spain	20 (M: 8, F: 12)	55 (range: 26 - 75)	70.6 ± 10.3§§	HC	X	4	Walnut	40 – 65g/day (~18% energy)	No nuts	cholesterol lowering Mediterranean diet (isocaloric)
Sauder et al. (2015)[14], United States	30 (M: 15, F: 15)	56.1 ± 7.8	31.2 ± 3.1	T2DM	X	4	Pistachio	20% total energy	Therapeutic Lifestyle Changes diet	Therapeutic Lifestyle Changes diet (isocaloric)
Sola et al. (2012)[42], Spain	56 (M: 23, F: 33)	*I:* 56.79 ± 10.46 *C:* 49.79 ± 9.53	*I:* 27.30 ± 3.01 *C:* 28.31 ± 3.25	Pre-HT or HT with at least one risk factor for CVD	P	4	Hazelnut	30g/day (in cocoa cream product)	Cocoa cream product*	Low saturated fat diet (isocaloric)
Sweazea et al. (2014)[43], United States	21 (M: 9, F: 12)	*I:* 57.8 ± 5.6 *C:* 54.7 ± 8.9	*I:* 37.2 ± 7.8 *C:* 33.5 ± 8.8	T2DM	P	12	Almond	43g (5-7 x week)	≤ 2 servings non-trial nuts/week	Habitual diet
Tey et al. (2014)[13], New Zealand	107 (M: 46, F: 61)	42.5 ± 12.4	30.6 ± 5.1	Overweight	P	12	Hazelnut	1. 30g/day 2. 60g/day	No nuts	Habitual diet
West et al. (2012)[54], United States	28 (M: 10, F: 18)	48 (SEM: 1.5)	26.8 (SEM: 0.7)	HL	X	4	Pistachio	1. 10% energy 2. 20% energy	NCEP Step 1 diet	Isocaloric diet
Wu et al. (2014)[55], Germany	40 (M: 10, F: 30)	60 ± 1	24.9 ± 0.6	Healthy (including overweight)	X	8	Walnut	43g/day	No nuts	Western diet with walnuts substituted for saturated fat (isocaloric)

*Study included other intervention group which was not relevant to this review, therefore this group was not included in this analysis

†Treated as comparison group for this analysis

‡ICAM [41]

•Adiponectin [40]

§VCAM [38]

¶CRP, IL-6, TNF-α [39]

**Gender breakdown estimated from % males reported in paper

††Characteristics reported for randomised participants

‡‡Gender breakdown for analysed participants not available

••Participants were randomised to one of two parallel groups (ad libitum or calorie adjusted). Within each group participants completed a 'walnut included' and 'walnut excluded' period in a cross-over design

§§ Body weight (kg) is reported when BMI was not available

¶¶ Mean intake

Abbreviations: BMI: body mass index; CAD: coronary artery disease; CHD: coronary heart disease; CI: confidence intervals; CVD: cardiovascular disease; DL: dyslipidaemia; F: female; HL: hyperlipidaemia; HT: hypertension; M: male; MetS: metabolic syndrome; NCEP: National Cholesterol Education Program; P: parallel; SEM: standard error of mean; T2DM: type 2 diabetes mellitus; X: cross-over

Effect of nut consumption on study outcomes

FMD

A total of nine strata from eight studies[14 17 18 22 35 49 54 56] explored the effect of nut consumption on FMD. The meta- analysis showed that nut consumption was associated with a significant increase in FMD (Figure 2 and Table 2). Sensitivity analyses indicated that excluding any one study did not substantially alter the effect (data not shown). The effect estimate was also similar after using different correlation coefficients (CC: 0.5, Supplementary material 3; CC: 0.25 and 0.75, data not shown). No significant differences were found for sub-group analyses (Supplementary material 4) although it was noted that only studies using walnuts found significant improvements in FMD.

CRP

A total of 26 strata from 25 studies[13-16 18 19 21 30 31 35-37 39 42-47 49-53 55] explored the effect of nut consumption on CRP. When all studies were included in the meta-analysis, nut consumption resulted in non-significant changes in CRP (Figure 3 and Table 2). The overall effect was relatively unchanged when studies with imputed standard deviations were removed from the analysis (Table 2). Sensitivity analyses identified two studies[15 47] that contributed substantially to the pooled result, as when they were excluded from the meta-analysis, the reductions in CRP were significant (Supplementary material 5).In addition, the use of different correlation coefficients did not change the overall effect found (CC: 0.5, Supplementary material 3; CC: 0.25 and 0.75, data not shown). Of all the sub-group analyses, statistically significant differences were only found between studies which included the energy value of nuts in the prescribed diet compared to those that did not (Supplementary material 4). An effect estimate of

-0.23 [-0.44, -0.01] was found for studies in which diets incorporated the energy value of nuts, whilst an effect estimate of -0.00 [-0.06, 0.05) was found for studies which did not ($Chi^2 = 3.99$, df = 1 (P = 0.05), $I^2 = 74.9\%$). However, when either of the studies identified in the sensitivity analysis[47,15] were excluded, this sub-group analysis no longer produced significant results (data not shown).

Adiponectin, TNF-α, IL-6, ICAM-1, VCAM-1

The meta- analysis showed that consumption of nuts did not result in significant changes in adiponectin, TNF-α, IL-6, ICAM-1, or VCAM-1 (Table 2 and Supplementary material 6). In the case that pooled analyses featured studies with imputed standard deviations (IL-6, ICAM-1, VCAM-1), excluding these studies did not substantially change the effect estimates (Table 2). Sensitivity analyses indicated that excluding any one study did not substantially alter the effect (data not shown). Overall effects also did not change when different correlation coefficients were used for cross-over studies (CC: 0.5, Supplementary material 3; CC: 0.25 and 0.75, data not shown). No significant differences between sub-groups were observed (Supplementary material 4).

Table 2: Changes in FMD, CRP, adiponectin, TNF-α, IL-6, ICAM-1, and VCAM-1following nut consumption, compared to control.

Outcome	Analysis description	Number of studies	Number of strata	Number of participants	Effect estimate	Inconsistency (I^2)
FMD (%)	**All studies‡**	8	9	652	0.79 [0.35, 1.23], P<0.001	0%
CRP (mg/L)	**All studies**	25	26	1578	-0.01 [-0.06, 0.03], P = 0.59†	20%
	Imputed SD excluded*	19	20	1244	-0.01 [-0.06, 0.04], P = 0.71	26%
Total adiponectin (ug/mL)	**All studies‡**	7	7	506	0.29 [-0.63, 1.21], P = 0.53	79%
TNF-α (pg/mL)	**All studies‡**	8	8	482	-0.05 [-0.13, 0.02], P = 0.17	2%
IL-6 (pg/mL)	**All studies**	13	13	906	-0.02 [-0.12, 0.08], P = 0.65,	10%
	Imputed SD excluded	11	11	800	-0.09 [-0.23, 0.05], P = 0.19	0%
ICAM-1	**All studies**	14	15	1047	0.68 [-0.53, 1.89], P = 0.27	0%

(ng/mL)	**Imputed SD excluded**	13	14	1011	0.68 [-0.53, 1.89], P = 0.27	0%
VCAM-1 (ng/mL)	**All studies**	13	14	804	2.83 [-8.85, 14.51], P = 0.63	0%
	Imputed SD excluded	12	13	768	2.43 [-9.29, 14.15], P = 0.68	0%

*Sensitivity analysis where studies with an imputed standard deviation were excluded

†Sensitivity analyses indicated that exclusion of either of two studies[15 47] resulted in an effect estimate of -0.22 [-0.40, -0.04].

‡No studies reporting FMD, adiponectin or TNF-α, required imputation of standard deviation

Publication bias

Funnel plots were generated for outcomes with ten or more strata (CRP, IL-6, ICAM-1, and VCAM-1) (Supplementary material 7). Egger's test indicated the presence of asymmetry in funnel plots for CRP (bias = -0.68 [95% CI = -1.06 to -0.30], P = 0.001) and IL-6 (bias = -0.72 [95% CI = -1.27 to -0.17], P = 0.0155), suggesting the possibility of publication bias. Funnel plot asymmetry was not detected for ICAM-1 or VCAM-1 (data not shown).

Risk of bias and quality of the body of evidence

The risk of bias was determined for each strata using the Cochrane Risk of Bias Tool and the results of the assessment are shown in Figure 4 and Supplementary material 8. The quality of the evidence was 'high' for FMD, ICAM-1, and VCAM-1. The quality was downgraded to 'moderate' for TNF-α due to risk of bias, and to 'low' for CRP and IL-6 due to both risk of bias and the likelihood of publication bias. The quality of the evidence for adiponectin was downgraded to 'very low' due to risk of bias, inconsistency, and imprecision (Supplementary material 9).

DISCUSSION

This systematic review and meta-analysis confirmed previously reported evidence[57] that consumption of nuts has favourable effects on FMD. With a high quality body of evidence and most studies relating to walnuts, the present review supports the 2011 conclusion of the European Food Safety Authority (EFSA) that walnut consumption improved endothelium-dependent vasodilation [57]. A meta-analysis was not part of the EFSA report[57], but the present study provides a meta-analysis that includes more recently published research[17 56]. It also

includes studies investigating other types of nuts[14 35 49 54]. Sub-group analyses found significant improvements in FMD only in those studies using walnuts, although the test for sub-group differences did not reach statistical significance. This may have been the result of the small number of studies available for FMD.

There are a number of mechanisms by which nuts, and walnuts in particular, could improve FMD. FMD is a measure of endothelial dysfunction[58], a condition characterised by reduced availability of the vasodilator nitric oxide (NO)[59]. Nuts contain high levels of L-arginine[60], an amino acid which acts as a precursor to NO[61]. Walnuts in particular are rich in alpha-linolenic acid, a polyunsaturated fatty acid that has been suggested to increase membrane fluidity, thus also increasing nitric oxide synthesis and release[62]. The antioxidant content of nuts may also play a role in the improvements in endothelial function observed[9].

Our finding of no significant effects on inflammatory biomarkers CRP, TNF-α, IL-6, ICAM-1, VCAM-1, or the anti-inflammatory biomarker adiponectin reflects the body of evidence available at this time. There may be effects with CRP but characteristics of the study sample or design of the dietary intervention may influence the ability to detect these effects. Sensitivity analyses indicated that results may have been disproportionally influenced by a small number of studies. Exclusion of either one of two studies[15 47] resulted in the meta-analysis yielding significant reductions in CRP following nut intake, suggesting these two studies were responsible for the results found. This appears to be the result of low reported CRP values and correspondingly small standard errors, resulting in these studies receiving substantially higher weighting than other studies in the pooled analysis. The study sample may in part explain these findings, as the study by Burns-Whitmore et al.[47] was conducted in healthy lacto-ovo vegetarians. Consumption of a plant-based diet has been associated with decreased

inflammation[63]. In contrast, Lee et al.[15] explored the effect of nut consumption in individuals with Metabolic Syndrome, which is typically associated with elevated CRP levels[64]. Reported units were confirmed with study authors.

The findings of this review may also have been influenced by the design of the dietary interventions included. Several studies[31 38-41] compared intake of nuts to a control intervention which also had the potential to influence inflammation and endothelial function, for example olive oil[65]. The potential impact of control groups on underestimating intervention effects has previously been highlighted in the weight loss literature[66]. Furthermore, whether the energy value of nuts was adjusted for in the total diet may have influenced results. Sub-group analyses suggested significant effects on CRP were only found when the energy provided by nuts was accounted for either by dietary modelling or advice to substitute other foods for nuts. This aligns with a previous review by our group which highlighted the importance of considering total energy intake in trials examining the effect of vegetable intake on weight loss[67]. Trials aiming to explore the influence of specific foods on health outcomes must carefully consider the design of the dietary intervention and controls arms, to avoid increases in total energy intake which could skew results.

The heterogeneity in study design elements, particularly related to dietary intervention, may explain why reviews exploring the effects of nut consumption on inflammation have found varying results. Although including fewer studies than in our review, a recently published review by Mazidi et al.[23] also found non-significant changes in inflammatory biomarkers CRP, IL-6, adiponectin, ICAM-1, and VCAM-1, but they did find small increases in CRP. This review appeared to have a broader eligibility criteria which also included post-prandial studies and those exploring the effects of soy consumption, Mazidi et al.[23]. In another review Barbour et al.[68]

reported significant reductions in CRP following nut consumption. It should be noted however, that Barbour et al.[68] included studies where nut consumption was encouraged as part of a suite of favourable dietary changes not matched in control groups, meaning the effect of the nuts themselves could not be isolated. In these circumstances it may not be possible to show whether effects observed were the result of increases in nut intake, or the wider dietary changes occurring. We avoided this problem by excluding studies with a portfolio of dietary changes not matched in the control group, or by treating a comparable intervention group as the "control" (or comparator), as in the case of the PREDIMED study[24]. Nevertheless, nuts appear in healthy dietary patterns and we have previously shown that consumption of a healthy dietary pattern (many of which include habitual nut intake) results in significant reductions in CRP[69].

It should be noted that while the current analysis found favourable effects of nut consumption on a marker of endothelial dysfunction, the lack of evidence for effects on cell adhesion molecules VCAM-1 and ICAM-1 suggests changes in endothelial cell activation may not have occurred. Given that the inflammatory cytokines which characteristically induce endothelial cell activation (for example TNF-α and IL-6)[59] also appeared unchanged, the lack of change found for ICAM-1 and VCAM-1 is perhaps not surprising. More research on this cluster of molecules will be informative.

This review had a number of strengths. It used a systematic methodology following current guidelines for systematic reviews, including prospective registration, and used the Cochrane Risk of Bias tool and GRADE method to evaluate the quality of evidence. We considered a range of biomarkers associated with inflammation and endothelial function, including the anti-inflammatory adipocyte adiponectin. The relatively small evidence base can be considered to be a limitation of this research. Variation also existed as a result of participant health status, nut type

and dose, and study duration, although these factors were explored in sub-group analyses. Background diets also varied between studies, with some studies prescribing diets based on dietary guidelines, whereas others allowed participants to follow their habitual diet. Analysis of funnel plots suggested the possibility of publication bias in the evidence base for CRP and IL-6, which resulted in downgrading the quality of the evidence for these outcomes. These findings suggest the need for more research in this area, with a particular focus on the registration of study protocols with detailed information on primary and secondary outcomes, to reduce the potential for publication bias.

This systematic review and meta-analysis of the effects of nut consumption on inflammation and endothelial function found evidence for favourable effects on FMD, a measure of endothelial function. Non-significant changes in CRP, adiponectin, TNF-α, IL-6, ICAM-1, VCAM-1 suggest a lack of consistent available evidence for effects of nut consumption on inflammation, although the results for CRP should be interpreted with caution due to the large influence of single studies on the pooled results. The findings of this analysis suggest a need for more research in this area, with a particular focus on randomised controlled trials incorporating the energy value of nuts into the total diet. There is also a need for appropriate dietary controls, and for the transparent registration of trial protocols.

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Access to data available on request (elizan@uow.edu.au)

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Figure titles:

Figure 1: PRISMA[25] flow diagram of study selection

Figure 2: Change in FMD (%) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals.

Figure 3: Change in C-reactive protein (mg/L) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals.

Figure 4: Risk of bias assessment as proportion of total strata.

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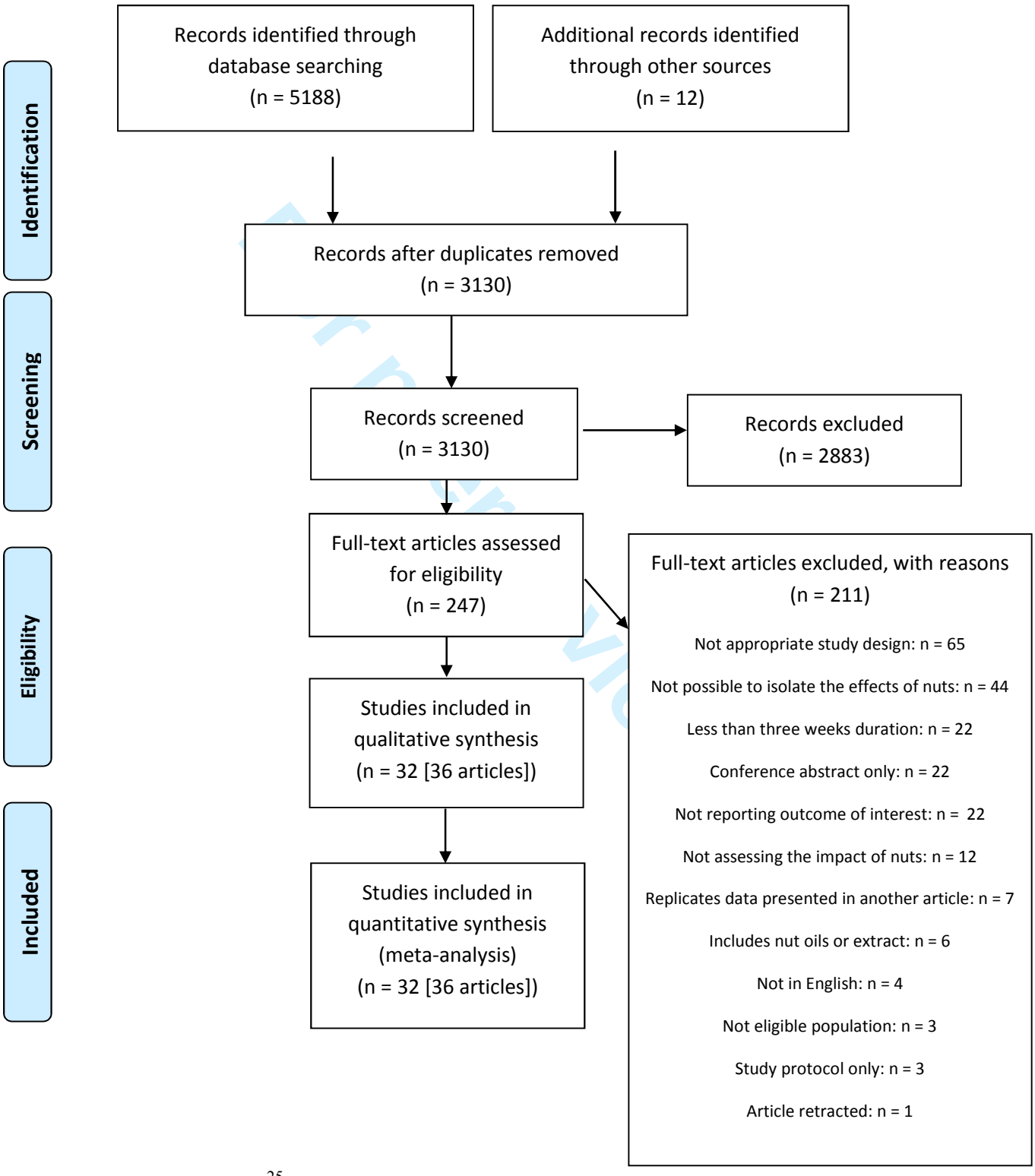

Figure 1: PRISMA[25] flow diagram of study selection

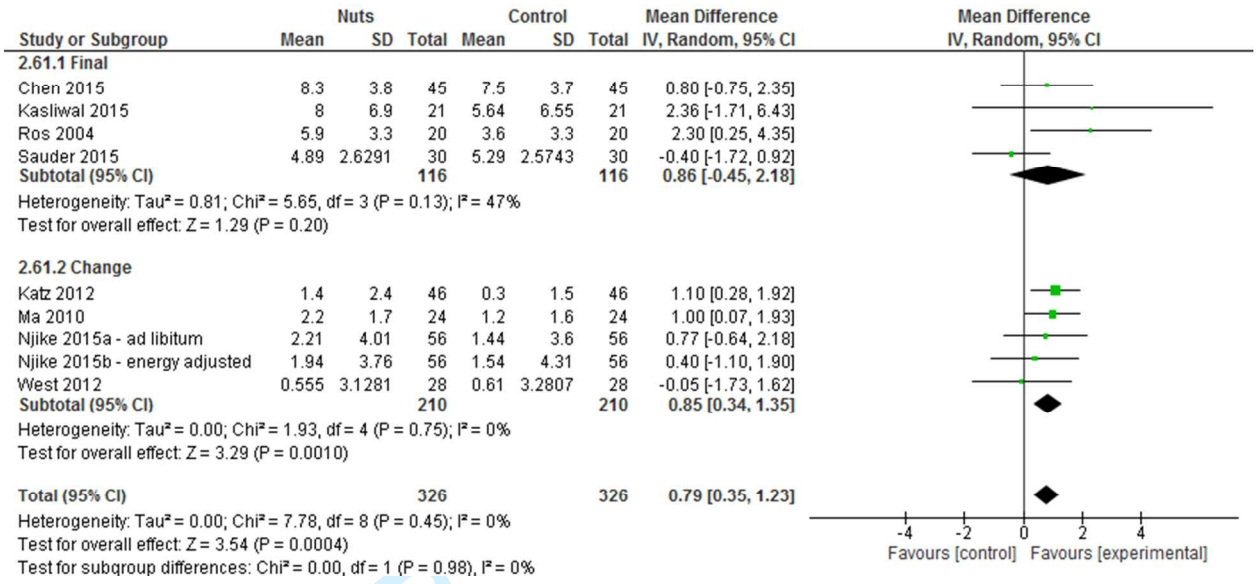

Figure 2: Change in FMD (%) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals.

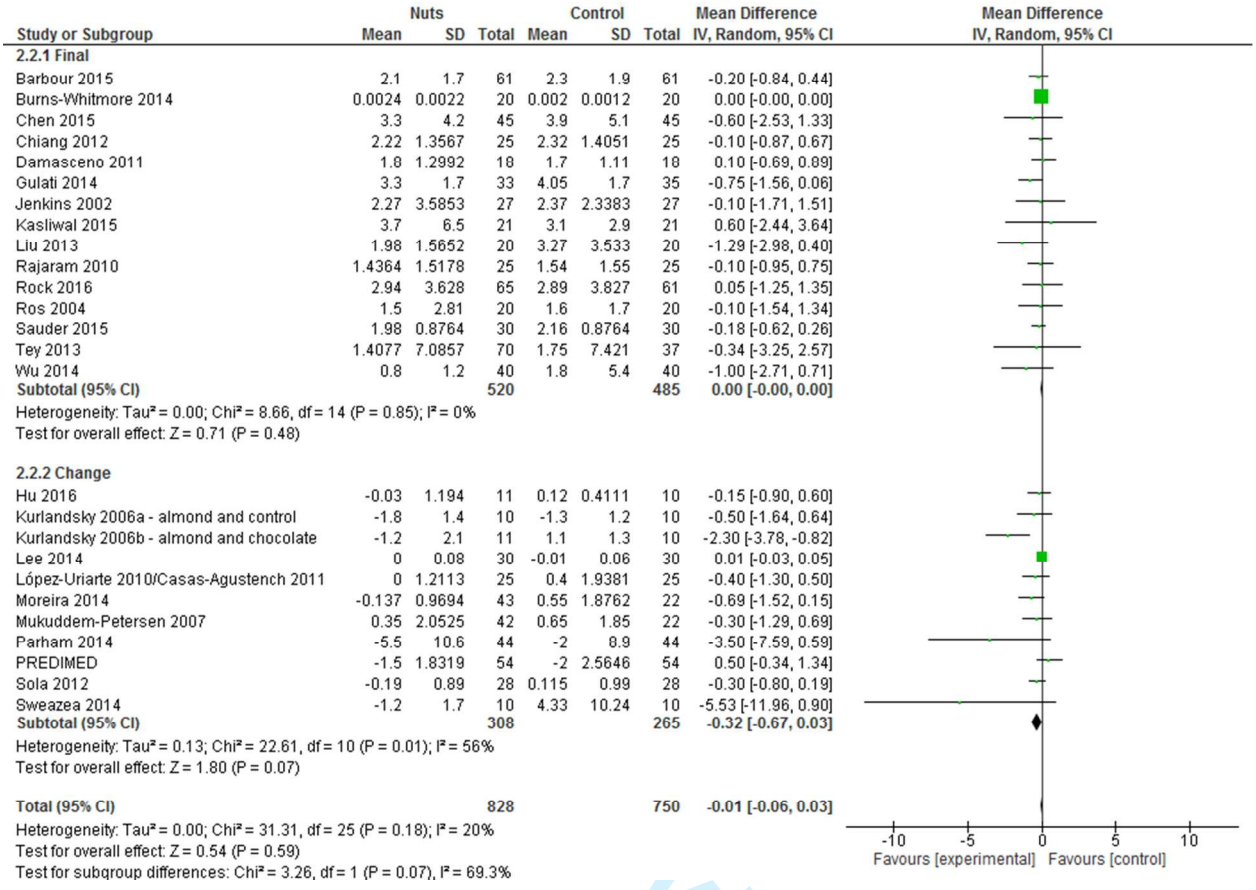

Figure 3: Change in C-reactive protein (mg/L) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals.

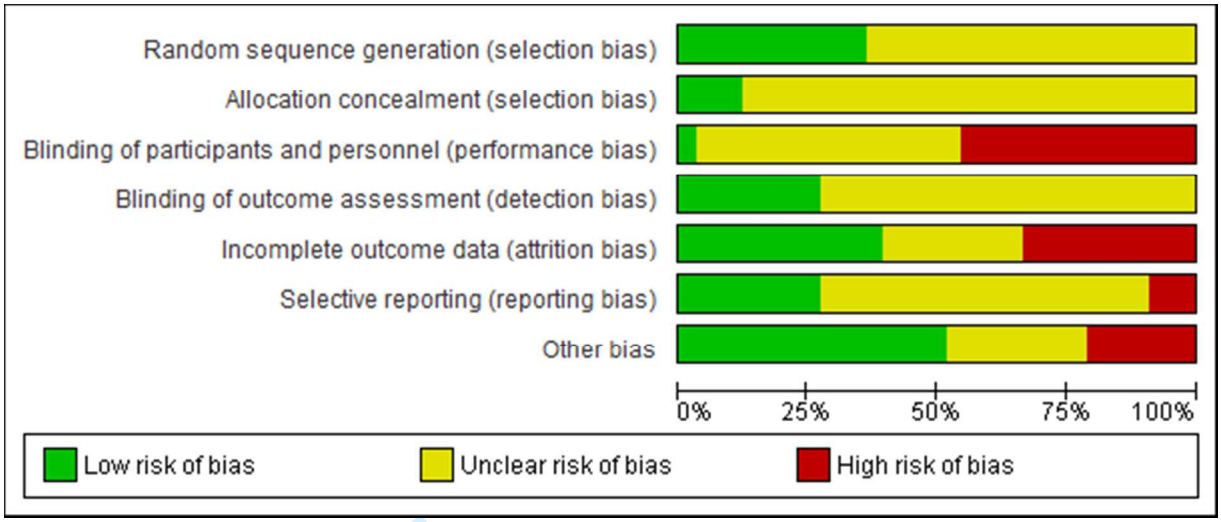

Figure 4: Risk of bias assessment as proportion of total strata.

List of supplementary material

Supplementary material 1: PRISMA checklist (as separate file)

Supplementary material 2: Example search strategy

Supplementary material 3: Forest plots of change in CRP after exclusion of individual studies

Supplementary material 4: Changes in CRP, adiponectin, TNF-α, IL-6, ICAM-1, VCAM-1, and FMD following nut consumption, compared to control, using correlation coefficient of 0.5

Supplementary material 5: Results of sub-group analyses

Supplementary material 6: Forest plots of change in biomarkers between nut consumption and control

Supplementary material 7: Funnel plots

Supplementary material 8: Risk of bias assessment

Supplementary material 9: GRADE assessment of the quality of the body of evidence

Supplementary material 2:

Search strategy: PubMed

((((((((((((((((((((((("nuts"[MeSH Terms]) OR nut) OR nuts) OR "juglans"[MeSH Terms]) OR walnut*) OR "prunus dulcis"[MeSH Terms]) OR almond*) OR "bertholletia"[MeSH Terms]) OR brazil nut*) OR Amazonia) OR "anacardium"[MeSH Terms]) OR cashew*) OR "corylus"[MeSH Terms]) OR hazelnut*) OR "macadamia"[MeSH Terms]) OR macadamia*) OR "carya"[MeSH Terms]) OR pecan*) OR "pinus"[MeSH Terms]) OR pine nut*) OR "pistacia"[MeSH Terms]) OR pistachio*) OR "arachis"[MeSH Terms]) OR peanut*))

AND

((((((((((((((((((("inflammation"[MeSH Terms]) OR inflammat*) OR endothelial*) OR "adiponectin"[MeSH Terms]) OR adiponectin) OR high molecular weight adiponectin) OR "c reactive protein"[MeSH Terms]) OR c reactive protein) OR c-reactive protein) OR CRP) OR "tumor necrosis factor alpha"[MeSH Terms]) OR tumor necrosis factor*) OR tumour necrosis factor*) OR TNF*) OR "interleukins"[MeSH Terms]) OR interleukin*) OR "cell adhesion molecules"[MeSH Terms]) OR adhesion molecule*) OR flow mediated dilat*) OR flow-mediated dilat*) OR FMD) OR "cytokines"[MeSH Terms]) OR cytokine*)

Supplementary material 3: Forest plots of change in CRP after exclusion of individual studies

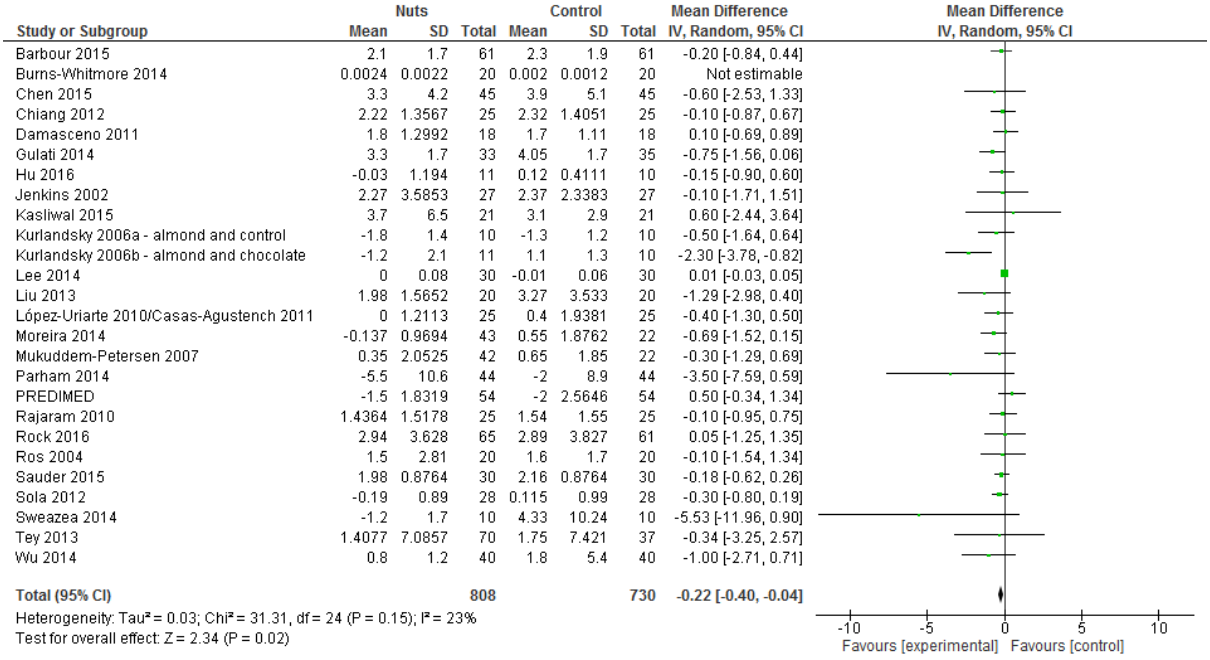

Figure 1: Change in CRP (mg/L) between nut consumption and control, after exclusion of Burns-Whitmore et al. (2014). Diamond indicates weighted mean difference with 95% confidence intervals.

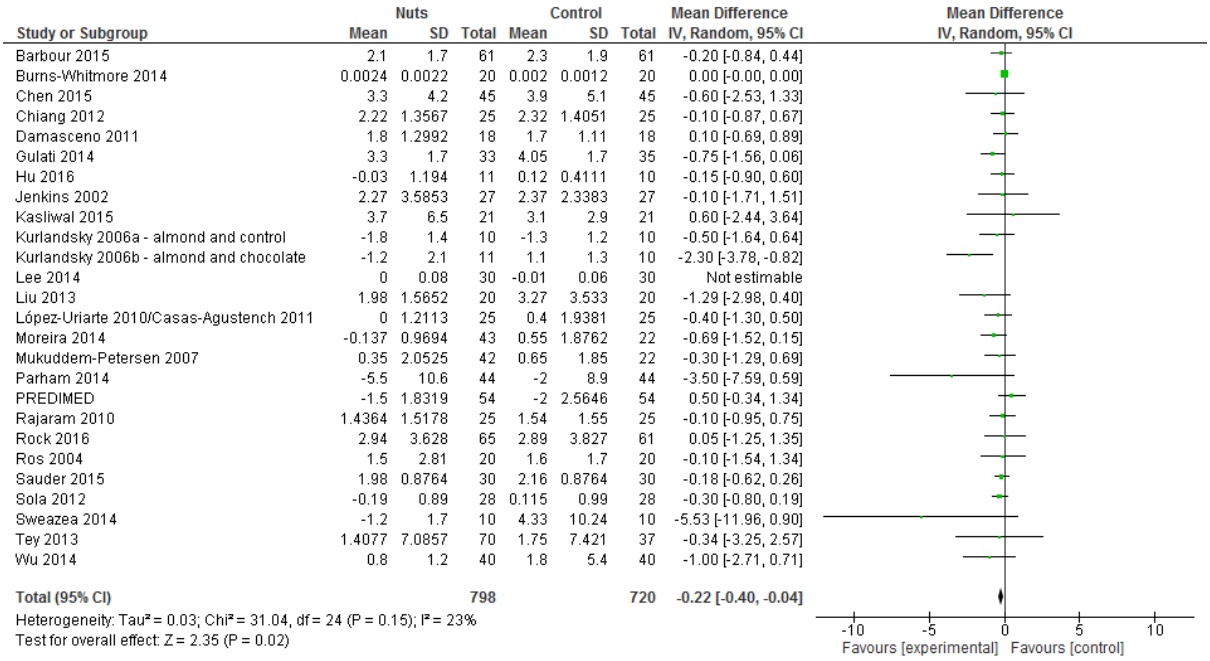

Figure 2: Change in CRP (mg/L) between nut consumption and control, after exclusion of Lee et al. (2014). Diamond indicates weighted mean difference with 95% confidence intervals.

Supplementary material 4: Changes in CRP, adiponectin, TNF-α, IL-6, ICAM-1, VCAM-1, and FMD following nut consumption, compared to control, using correlation coefficient of 0.5

Outcome	Number of analyses	Number of participants	Effect estimate	Inconsistency (I^2)
CRP (mg/L)	26	1578	-0.03 [-0.09, 0.03], P = 0.30	33%
Total adiponectin (ug/mL)	7	506	0.15 [-0.77, 1.07], P = 0.75	81%
TNF-α (pg/mL)	8	482	-0.05 [-0.12, 0.02], P = 0.17	7%
IL-6 (pg/mL)	13	906	-0.06 [-0.16, 0.04], P = 0.24	28%
ICAM-1 (ng/mL)	15	1047	0.62 [-0.24, 1.49], P = 0.16	0%
VCAM-1 (ng/mL)	14	804	1.25 [-12.09, 14.59], P = 0.85	9%
FMD (%)	9	652	0.74 [0.27, 1.20], P = 0.002	46%

Supplementary material 5: Results of sub-group analyses

Table 1: Results of sub-group analyses for CRP

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	17	847	-0.00 [-0.04, 0.03]	Chi² = 1.02, df = 1 (P = 0.31), I² = 1.9%
	More than three months	9	731	-0.24 [-0.69, 0.22]	
Risk of bias	Low/unclear	11	588	-0.25 [-0.53, 0.04]	Chi² = 2.82, df = 1 (P = 0.09), I² = 64.6%
	High	15	990	0.00 [-0.00, 0.00]	
Nut type	Almond	7	295	-0.79 [-1.52, -0.06]	Chi² = 10.42, df = 6 (P = 0.11), I² = 42.4%
	Walnut	5	336	0.00 [-0.00, 0.00]	
	Hazelnut	2	163	-0.31 [-0.79, 0.18]	
	Mixed nut	5	318	0.01 [-0.03, 0.05]	
	Peanut	2	187	-0.38 [-0.89, 0.13]	

	Pistachio	4	258	-0.42 [-1.03, 0.19]	
	Brazil nut	1	21	-0.15 [-0.90, 0.60]	
Health status	Healthy	2	61	0.00 [-0.00, 0.00]	$Chi^2 = 10.41$, df = 5 (P = 0.06), I^2 = 52.0%
	Chronic disease risk factors	14	869	-0.29 [-0.54, -0.04]	
	T2DM	4	208	-1.18 [-2.70, 0.35]	
	MetS	4	242	-0.19 [-0.55, 0.17]	
	CAD	1	90	-0.60 [-2.53, 1.33]	
	Combination	1	108	0.50 [-0.34, 1.34]	
Energy value of nuts included in diet	Adjusted	16	1029	-0.23 [-0.44, -0.01]	$Chi^2 = 3.99$, df = 1 (P = 0.05), I^2 = 74.9%
	Not adjusted	10	549	-0.00 [-0.06, 0.05]	

Table 2: Results of sub-group analyses for FMD

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	6	386	0.77 [0.17,1.38]	Chi² = 0.01, df = 1 (P = 0.91), I² = 0%
	More than three months	3	266	0.70 [-0.29, 1.70]	
Risk of bias	Low/unclear	6	480	0.69 [0.22, 1.16]	Chi² = 1.32, df = 1 (P = 0.25), I² = 24.2%
	High	3	172	1.43 [0.25, 2.61]	
Nut type	Almond	1	90	0.80 [-0.75, 2.35]	Chi² = 3.86, df = 2 (P = 0.15), I² = 48.1%
	Walnut	5	404	1.02 [0.51, 1.53]	
	Pistachio	3	158	-0.11 [-1.11, 0.90]	
Health status	Chronic disease risk factors	4	230	1.09 [0.25, 1.92]	Chi² = 0.97, df = 3 (P = 0.81), I² = 0%
	T2DM	2	108	0.38 [-0.98, 1.74]	

	CAD	1	90	0.80 [-0.75, 2.35]	
	Combination	2	224	0.60 [-0.43, 1.62]	
Energy value of nuts included in diet	Adjusted	8	540	0.77 [0.27, 1.27]	$Chi^2 = 0.00$, df = 1 (P = 1.00), I^2 = 0%
	Not adjusted	1	112	0.77 [-0.64, 2.18]	

Table 3: Results of sub-group analyses for adiponectin

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	2	130	-0.60 [-2.48, 1.28]	Chi² = 1.03, df = 1 (P = 0.31), I² = 3.3%
	More than three months	5	376	1.71 [-2.33, 5.75]	
Risk of bias	Low/unclear	3	234	-0.00 [-0.00, 0.00]	Chi² = 0.45, df = 1 (P = 0.50), I² = 0%
	High	4	272	1.91 [-3.70, 7.53]	
Nut type	Walnut	2	96	-0.52 [-3.78, 2.75]	Chi² = 0.57, df = 2 (P = 0.75), I² = 0%
	Mixed nut	3	234	-0.00 [-0.00, 0.00]	
	Pistachio	2	176	4.49 [-8.30, 17.28]	
Health status	Chronic disease risk factors	2	178	-2.33 [-5.28, 0.63]	Chi² = 3.42, df = 2 (P = 0.18), I² = 41.5%
	MetS	3	178	0.53 [-0.49, 1.55]	

	Combination	2	150	-2.05 [-11.64, 7.54]	
Energy value of nuts included in diet	Adjusted	5	396	0.80 [-4.62, 6.22]	$Chi^2 = 0.08$, df = 1 (P = 0.77), I^2 = 0%
	Not adjusted	2	110	-0.00 [-0.00, 0.00]	

Table 4: Results of sub-group analyses for TNF-α

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	5	285	-0.06 [-0.12, 0.01]	Chi² = 0.21, df = 1 (P = 0.65), I² = 0%
	More than three months	3	197	-0.70 [-3.48, 2.08]	
Risk of bias	Low/unclear	2	148	0.11 [-0.51, 0.73]	Chi² = 0.21, df = 1 (P = 0.65), I² = 0%
	High	6	334	-0.04 [-0.22, 0.15]	
Nut type	Almond	3	151	-0.06 [-0.13, 0.01]	Chi² = 6.75, df = 4 (P = 0.15), I² = 40.8%
	Walnut	2	90	-0.03 [-0.21, 0.14]	
	Mixed nut	1	108	0.70 [-0.41, 1.81]	
	Peanut	1	65	-0.16 [-1.41, 1.10]	
	Pistachio	1	68	-3.70 [-6.93, -0.47]	
Health status	Healthy	1	40	-0.01 [-0.24, 0.22]	Chi² = 7.08, df = 5 (P = 0.21), I² =

	Chronic disease risk factors	2	115	-0.07 [-0.34, 0.20]	29.4%
	T2DM	2	61	-0.06 [-0.13, 0.01]	
	MetS	1	68	-3.70 [-6.93, -0.47]	
	CAD	1	90	0.10 [-0.54, 0.74]	
	Combination	1	108	0.70 [-0.41, 1.81]	
Energy value of nuts included in diet	Adjusted	6	421	-0.04 [-0.24, 0.15]	$Chi^2 = 0.05$, df = 1 (P = 0.83), I^2 = 0%
	Not adjusted	2	61	-0.01 [-0.24, 0.22]	

Table 5: Results of sub-group analyses for IL-6

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	7	386	0.04 [-0.02, 0.09]	Chi² = 2.71, df = 1 (P = 0.10), I² = 63.1%
	More than three months	6	520	-0.19 [-0.45, 0.07]	
Risk of bias	Low/unclear	5	314	-0.01 [-0.26, 0.23]	Chi² = 0.62, df = 1 (P = 0.43), I² = 0%
	High	8	592	-0.13 [-0.29, 0.03]	
Nut type	Almond	4	201	-0.16 [-0.44, 0.13]	Chi² = 5.17, df = 4 (P = 0.27), I² = 22.6%
	Walnut	3	216	-0.11 [-0.31, 0.10]	
	Hazelnut	2	163	0.05 [-0.01, 0.11]	
	Mixed nut	3	218	-0.18 [-0.99, 0.63]	
	Pistachio	1	108	-0.14 [-0.47, 0.19]	
Health status	Chronic disease risk	6	497	0.04 [-0.02, 0.10]	Chi² = 3.09, df = 5 (P = 0.69), I² = 0%

	factors				
	Healthy	1	40	-0.10 [-0.39, 0.19]	
	MetS	2	110	-0.47 [-2.44, 1.49]	
	T2DM	2	61	-0.14 [-0.46, 0.18]	
	CAD	1	90	-0.50 [-1.62, 0.62]	
	Combination	1	108	0.00 [-0.41, 0.41]	
Energy value of nuts included in diet	Adjusted	8	628	0.03 [-0.02, 0.09]	$Chi^2 = 0.68$, df = 1 (P = 0.41), I^2 = 0%
	Not adjusted	5	278	-0.18 [-0.68, 0.32]	

Table 6: Results of sub-group analyses for ICAM-1

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	12	537	0.66 [-0.56, 1.88]	Chi² = 0.04, df = 1 (P = 0.83), I² = 0%
	More than three months	3	510	2.35 [-13.26, 17.96]	
Risk of bias	Low/unclear	8	660	4.58 [-2.68, 11.85]	Chi² = 1.14, df = 1 (P = 0.29), I² = 12.4%
	High	7	387	0.57 [-0.66, 1.80]	
Nut type	Almond	3	81	11.65 [-1.49, 24.80]	Chi² = 3.34, df = 4 (P = 0.50), I² = 0%
	Walnut	5	244	0.58 [-0.65, 1.81]	
	Hazelnut	2	163	-3.32 [-22.42, 15.78]	
	Mixed nut	4	499	3.75 [-7.31, 14.81]	
	Pistachio	1	60	-2.60 [-18.13, 12.93]	
Health status	Healthy	1	40	0.65 [-0.59, 1.89]	Chi² = 1.02, df = 4 (P = 0.91), I² =

	Chronic disease risk factors	9	444	0.86 [-6.94, 8.65]	0%
	T2DM	2	100	-1.67 [-16.50, 13.16]	
	MetS	2	110	-13.46 [-76.61, 49.70]	
	Combination	1	353	8.00 [-8.85, 24.85]	
Energy value of nuts included in diet	Adjusted	9	749	-1.31 [-8.90, 6.29]	$Chi^2 = 0.48$, df = 1 (P = 0.49), I^2 = 0%
	Not adjusted	6	298	2.06 [-3.72, 7.84]	

Table 7: Results of sub-group analyses for VCAM-1

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	11	537	2.23 [-9.68, 14.13]	Chi² = 0.02, df = 1 (P = 0.89), I² = 0%
	More than three months	3	267	-4.16 [-96.76, 88.44]	
Risk of bias	Low/unclear	8	417	2.39 [-9.72, 14.50]	Chi² = 0.04, df = 1 (P = 0.83), I² = 0%
	High	6	387	7.42 [-38.20, 53.04]	
Nut type	Almond	4	171	1.11 [-13.10, 15.33]	Chi² = 1.56, df = 4 (P = 0.82), I² = 0%
	Walnut	3	154	-30.19 [-99.92, 39.53]	
	Hazelnut	2	163	17.62 [-24.61, 59.85]	
	Mixed nut	4	256	9.30 [-21.20, 39.80]	
	Pistachio	1	60	3.40 [-60.84, 67.64]	
Health status	Chronic disease risk	8	394	3.95 [-9.12, 17.02]	Chi² = 2.08, df = 4 (P = 0.72), I² = 0%

	factors				
	T2DM	2	100	-17.58 [-67.98, 32.82]	
	MetS	2	110	9.61 [-23.37, 42.59]	
	CAD	1	90	-48.00 [-193.52, 97.52]	
	Combination	1	110	-70.00 [-230.43, 90.43]	
Energy value of nuts included in diet	Adjusted	9	546	-12.78 [-42.38, 16.83]	$Chi^2 = 1.27$, df = 1 (P = 0.26), I^2 = 21.0%
	Not adjusted	5	258	5.71 [-7.00, 18.42]	

Supplementary material 6: Forest plots of change in biomarkers between nut consumption and control

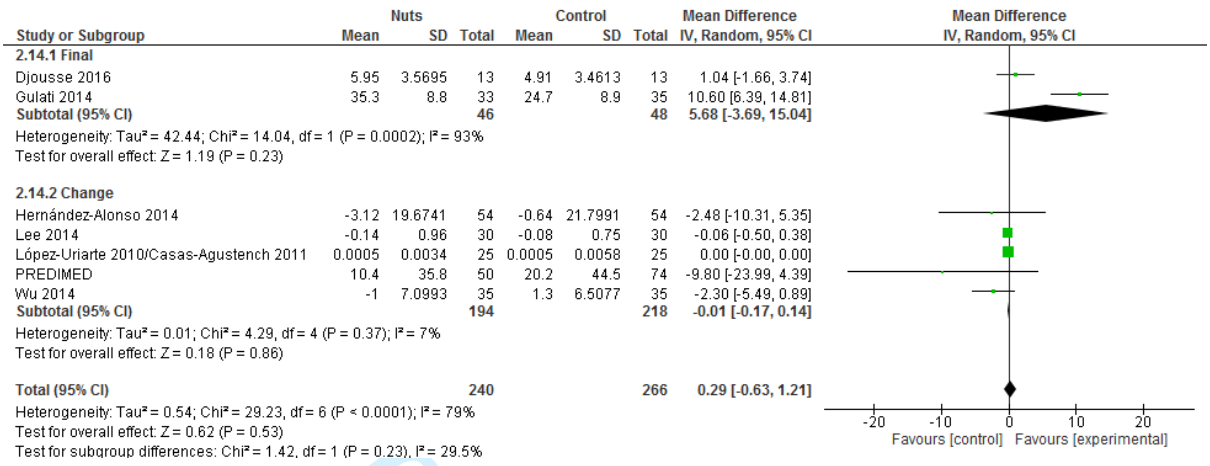

Figure 3: Change in adiponectin (ug/mL) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals.

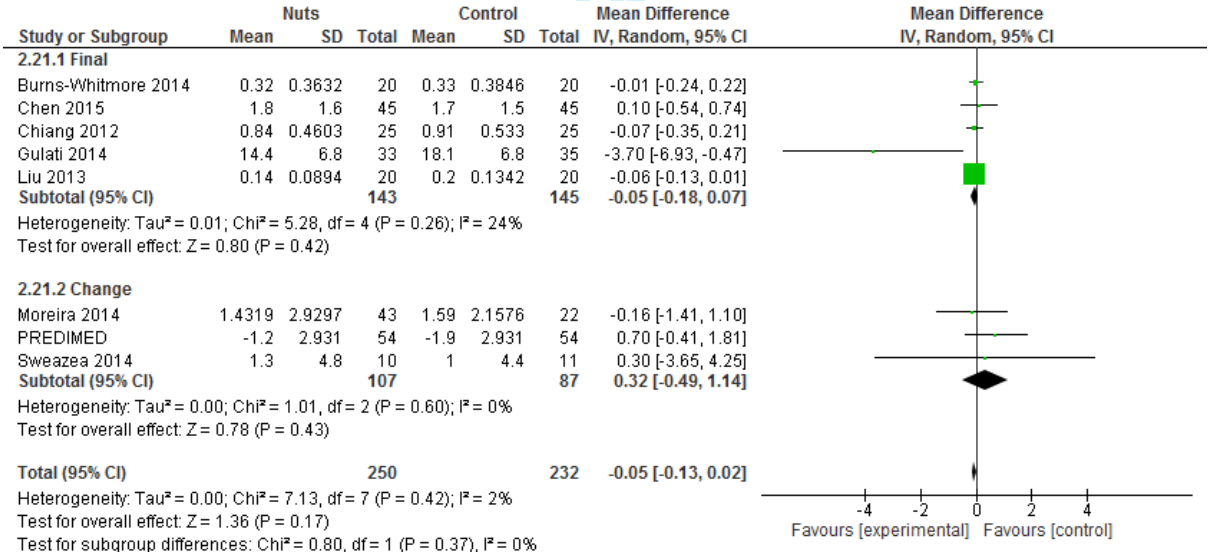

Figure 4: Change in TNF-α (pg/mL) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals.

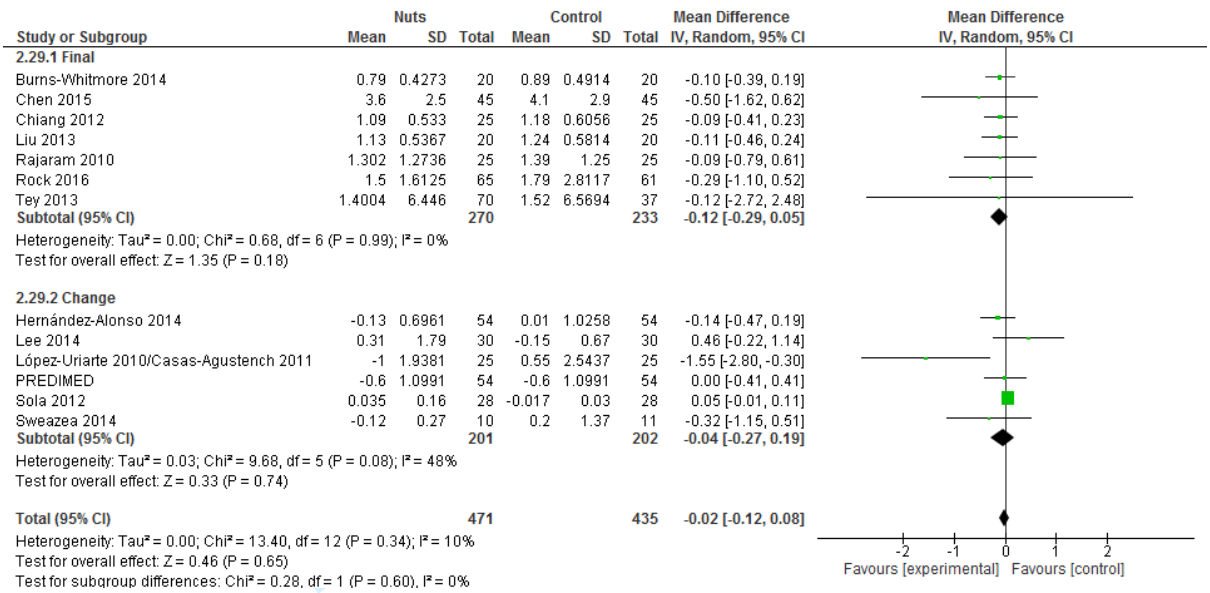

Figure 5: Change in IL-6 (pg/mL) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals

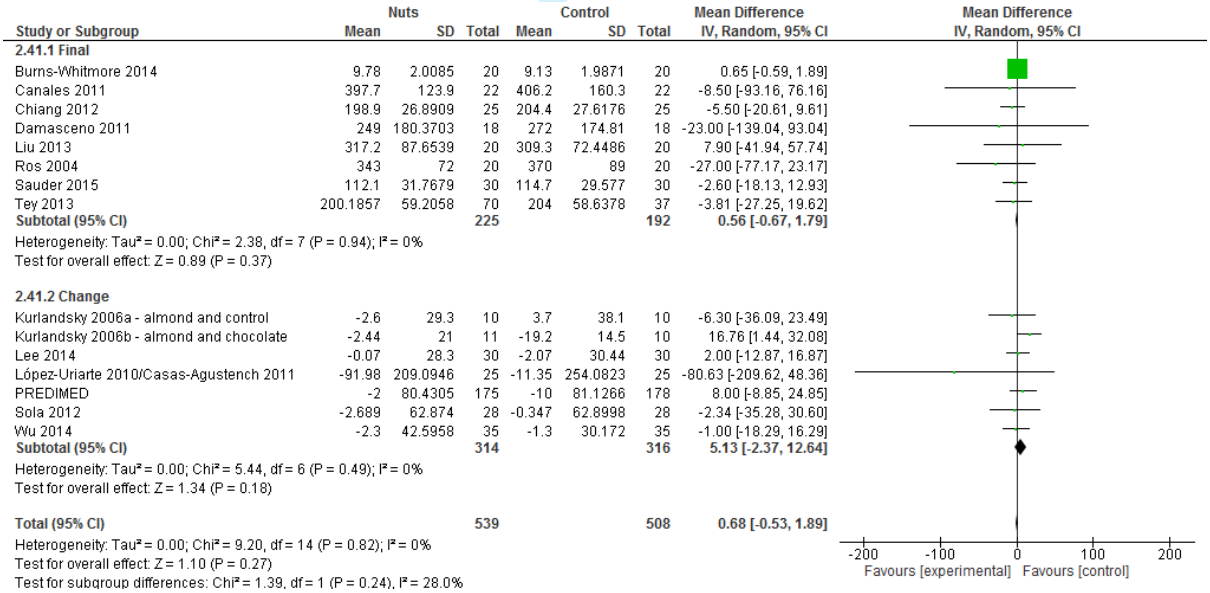

Figure 6: Change in ICAM-1 (ng/mL) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals

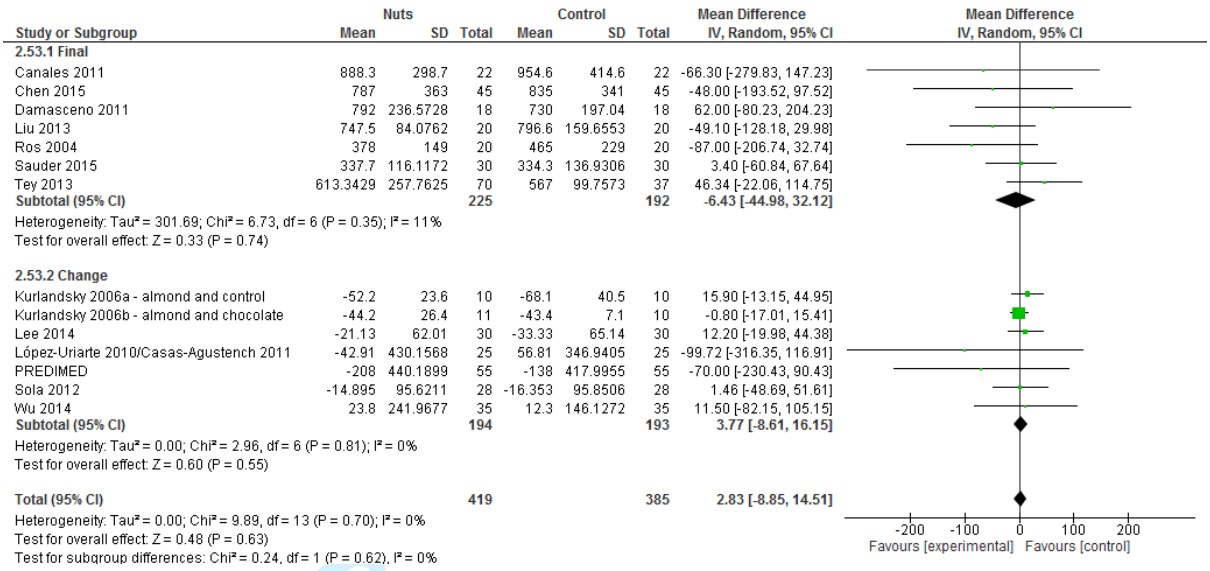

Figure 7: Change in VCAM-1 (ng/mL) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals

Supplementary material 7: Funnel plots

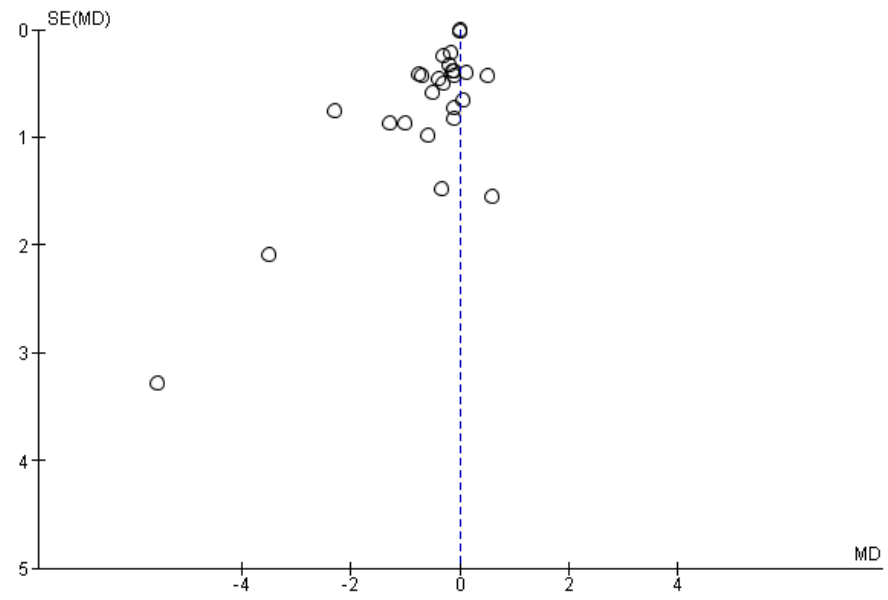

Figure 8: Funnel plot of the effect of nut consumption on CRP

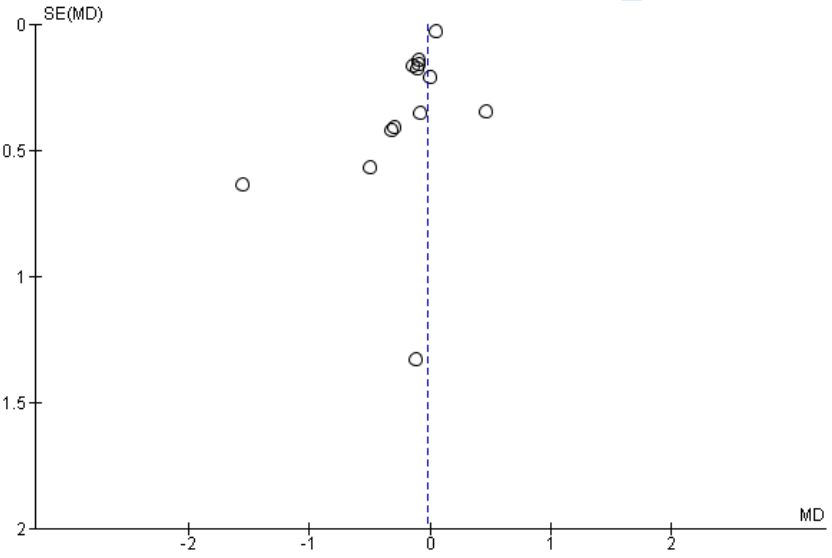

Figure 9: Funnel plot of the effect of nut consumption on IL-6

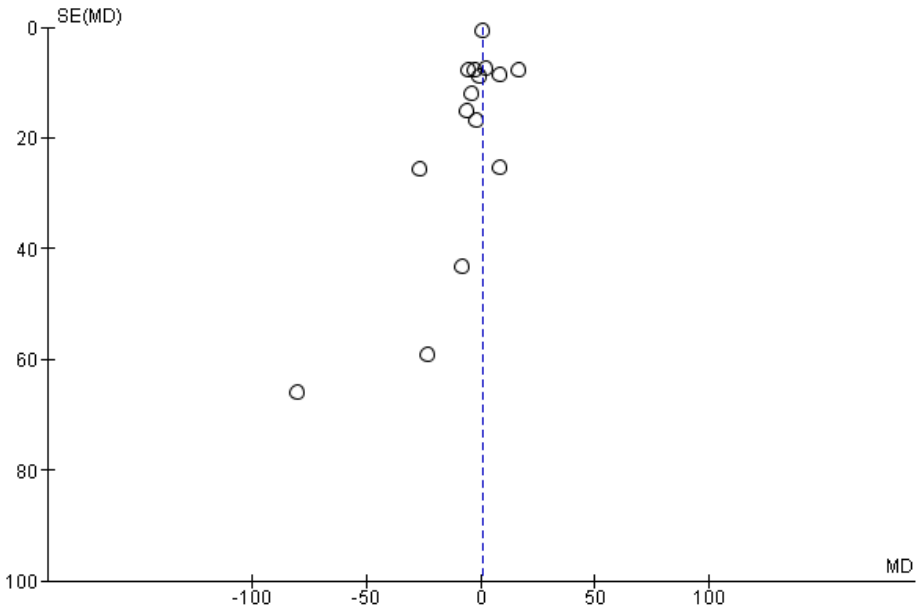

Figure 10: Funnel plot of the effect of nut consumption on ICAM-1

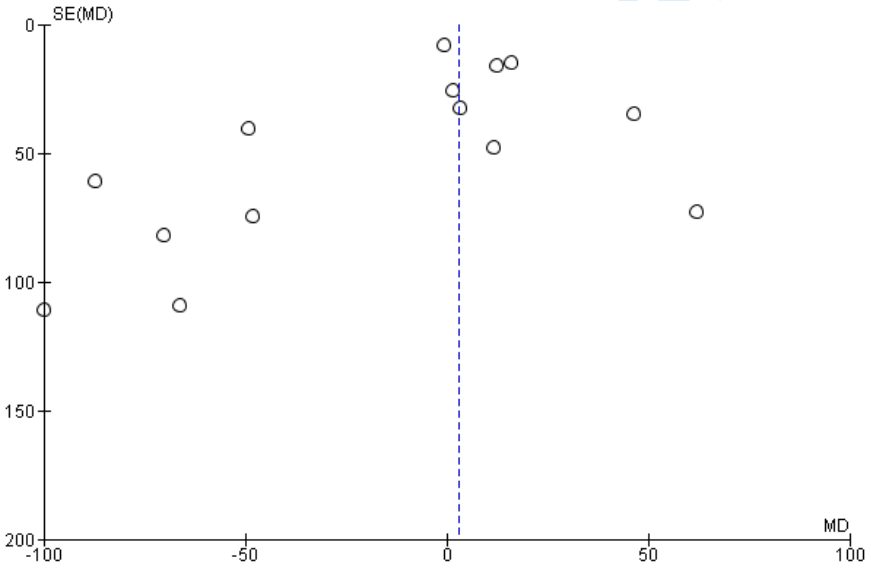

Figure 11: Funnel plot of the effect of nut consumption on VCAM-1

Supplementary material 8: Risk of bias assessment

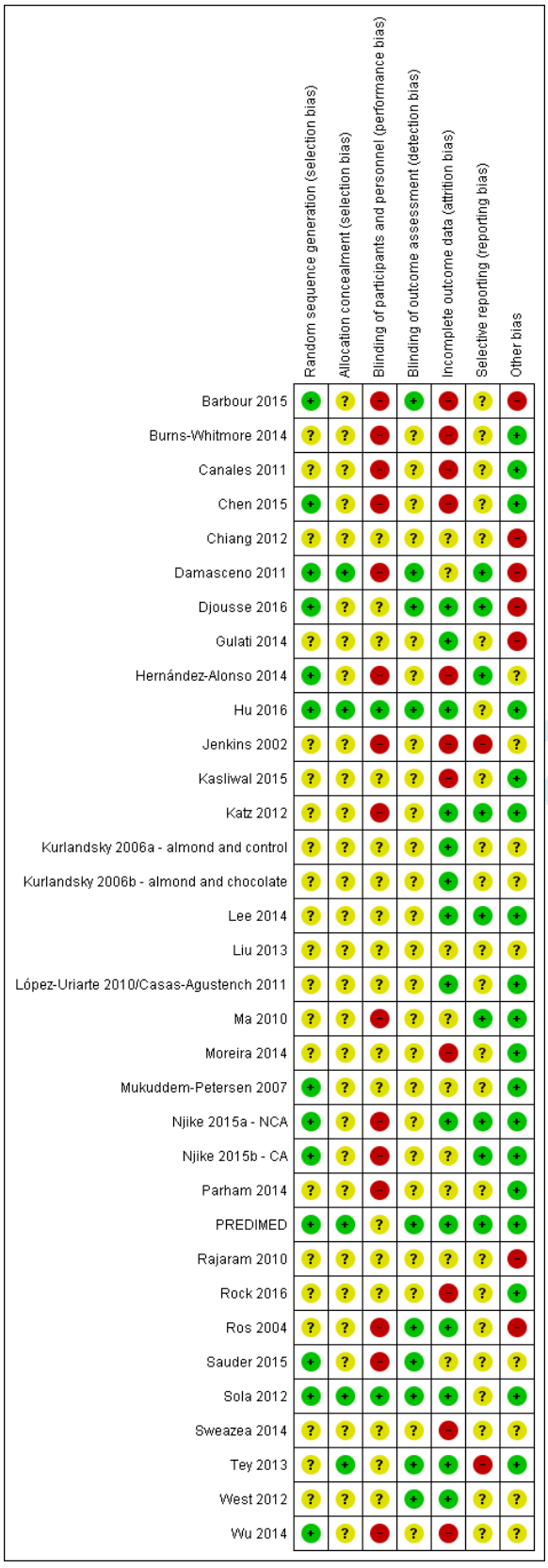

Figure 12: Risk of bias assessment for each study

Supplementary material 9: GRADE assessment of the quality of the body of evidence

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	nut consumption	control	Relative (95% CI)	Absolute (95% CI)		
CRP												
26	randomised trials	serious [a]	not serious [b]	not serious	not serious	publication bias strongly suspected [c]	828	750	-	MD **0.01 lower** (0.06 lower to 0.03 higher)	⊕⊕◯◯ LOW	IMPORTANT
Adiponectin												
7	randomised trials	serious [d]	serious [e]	not serious	serious [f]	none	240	266	-	MD **0.29 higher** (0.63 lower to 1.21 higher)	⊕◯◯◯ VERY LOW	IMPORTANT
TNF-a												
8	randomised trials	serious [g]	not serious	not serious	not serious	none	250	232	-	MD **0.05 lower** (0.13 lower to 0.02 higher)	⊕⊕⊕◯ MODERATE	IMPORTANT
IL-6												
13	randomised trials	serious [h]	not serious	not serious	not serious	publication bias strongly suspected [i]	471	435	-	MD **0.02 lower** (0.12 lower to 0.08 higher)	⊕⊕◯◯ LOW	IMPORTANT
ICAM-1												

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	nut consumption	control	Relative (95% CI)	Absolute (95% CI)		
15	randomised trials	not serious [i]	not serious	not serious	not serious	none	539	508	-	MD **0.68 higher** (0.53 lower to 1.89 higher)	⊕⊕⊕⊕ HIGH	IMPORTANT
VCAM-1												
14	randomised trials	not serious [k]	not serious	not serious	not serious	none	419	385	-	MD **2.83 higher** (8.85 lower to 14.51 higher)	⊕⊕⊕⊕ HIGH	IMPORTANT
FMD												
9	randomised trials	not serious [l]	not serious	not serious	not serious	none	326	326	-	MD **0.79 higher** (0.35 higher to 1.23 higher)	⊕⊕⊕⊕ HIGH	IMPORTANT

CI: Confidence interval; **MD:** Mean difference

a. The studies were viewed as being in the category of 'serious limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'high risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'high risk' needed to be categorised as either 'serious limitations' or 'very serious limitations'. In view of the potential implications of the 'high risk' aspects on the quality of the body of evidence, 'serious limitations' was selected

b. I squared value of 20%, indicating minimal heterogeneity

c. Funnel plot indicates likelihood of publication bias

d. The studies were viewed as being in the category of 'serious limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'high risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'high risk' needed to be categorised as either 'serious limitations' or 'very serious limitations'. In view of the potential implications of the 'high risk' aspects on the quality of the body of evidence, 'serious limitations' was selected

e. I squared value of 79% indicating considerable heterogeneity

f. Total sample size is greater than 400, however 95% CIs overlap no effect and include appreciable benefit or harm

g. The studies were viewed as being in the category of 'serious limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'high risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'high risk' needed to be categorised as either 'serious limitations' or 'very serious limitations'. In view of the potential implications of the 'high risk' aspects on the quality of the body of evidence, 'serious limitations' was selected

h. The studies were viewed as being in the category of 'serious limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'high risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'high risk' needed to be categorised as either 'serious limitations' or 'very serious limitations'. In view of the potential implications of the 'high risk' aspects on the quality of the body of evidence, 'serious limitations' was selected

i. Funnel plot indicates likelihood of publication bias

j. The studies were viewed as being in the category of 'no limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'unclear risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'unclear risk' needed to be categorised as either 'no limitations' or 'serious limitations'. In view of the potential implications of the 'unclear risk' aspects on the quality of the body of evidence, 'no limitations' was selected

k. The studies were viewed as being in the category of 'no limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'unclear risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'unclear risk' needed to be categorised as either 'no limitations' or 'serious limitations'. In view of the potential implications of the 'unclear risk' aspects on the quality of the body of evidence, 'no limitations' was selected

l. The studies were viewed as being in the category of 'no limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'unclear risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'unclear risk' needed to be categorised as either 'no limitations' or 'serious limitations'. In view of the potential implications of the 'unclear risk' aspects on the quality of the body of evidence, 'no limitations' was selected

Supplementary material 1: PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	2
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5 -6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5 -6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supplementary material 2
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6-7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6-7

Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7,8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7-8

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Supplementary material 8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Table 2, Figure 2, Figure 3, Supplementary material 6
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Table 2
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Figure 4
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Table 2, Supplementary material 3, 4, 5

DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	20 - 24
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	23 – 24
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	24
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	24

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

Page 2 of 2

BMJ Open

The effect of nut consumption on markers of inflammation and endothelial function: a systematic review and meta-analysis of randomised controlled trials

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Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Public health
Keywords:	nut, inflammation, endothelial function, flow mediated dilation, systematic review

Title: The effect of nut consumption on markers of inflammation and endothelial function: a systematic review and meta-analysis of randomised controlled trials

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Word count: 5159

Number of tables: 2

Number of figures: 4

The effect of nut consumption on markers of inflammation and endothelial function: a systematic review and meta-analysis of randomised controlled trials

Abstract

Objectives: To examine the effect of nut consumption on inflammatory biomarkers and endothelial function.

Design: A systematic review and meta-analysis

Data sources: Medline, PubMed, CINAHL and Cochrane Central Register of Controlled Trials (all years to 13 January 2017)

Eligibility criteria: Randomised controlled trials (with a duration of three weeks or more) or prospective cohort designs conducted in adults; studies assessing the effect of consumption of tree nuts or peanuts on C-reactive protein (CRP), adiponectin, tumour necrosis factor-alpha, interleukin-6, intercellular adhesion molecule 1, vascular cell adhesion protein 1, and flow mediated dilation (FMD).

Data extraction and analysis: Relevant data was extracted for summary tables and analyses by two independent researchers. Random effects meta-analyses were conducted to explore weighted mean differences (WMD) in change or final mean values for each outcome.

Results: A total of n=32 studies (all randomised controlled trials) were included in the review. The effect of nut consumption on FMD was explored in n=9 strata from n=8 studies (involving n=652 participants), with consumption of nuts resulting in significant improvements in FMD (WMD: 0.79% [95% CI: 0.35, 1.23]). Nut consumption resulted in small, non-significant differences in CRP (WMD: -0.01mg/L [95% CI: -0.06, 0.03]) (n=26 strata from n=25 studies), although sensitivity analyses suggest results for CRP may have been influenced by two

individual studies. Small, non-significant differences were also found for other biomarkers of inflammation.

Conclusions: This systematic review and meta-analysis of the effects of nut consumption on inflammation and endothelial function found evidence for favourable effects on FMD, a measure of endothelial function. Non-significant changes in other biomarkers indicate a lack of consistent evidence for effects of nut consumption on inflammation. The findings of this analysis suggest a need for more research in this area, with a particular focus on randomised controlled trials.

Review registration: CRD42016045424

Strengths and limitations of this study

- This is the first known systematic review and meta-analysis which examined the effect of nut consumption on inflammation and endothelial function, in studies which isolated the effect of nut consumption
- The protocol for the review was pre-registered, and the review followed the requirements of the PRISMA statement
- Risk of bias was assessed using the Cochrane Risk of Bias Tool, and the quality of the body of evidence was then determined using GRADE
- The available evidence base for some of the biomarkers explored was small
- There were variations in the included studies, such as participant health status, nut type and dose, and study duration, although these factors were explored in sub-group analyses

INTRODUCTION

Chronic conditions such as type 2 diabetes, and metabolic syndrome are known to be underpinned by a state of low-grade inflammation, which play a central role in disease progression, and in the development of atherosclerosis[1 2]. Changes in this inflammatory state can be identified via biomarkers of inflammation including C-reactive protein (CRP)[3], tumour necrosis factor-alpha (TNF-α)[4], interleukin-6 (IL-6)[5], and the adhesion molecules intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion protein 1 (VCAM-1)[6], as well as anti-inflammatory biomarkers such as the adipocyte adiponectin[7]. Endothelial dysfunction is a central component in the development and progression of atherosclerosis, with brachial flow mediated dilation (FMD), a non-invasive measure of endothelial function, found to be significantly associated with risk of cardiovascular events[8].

Given that markers of inflammation and endothelial function can indicate changes in disease development and progression, they can be used to explore the impact of consumption of specific foods on health. Nuts contain a wide range of nutrients and bioactive components which may moderate inflammation and the development of endothelial dysfunction, such as alpha-linolenic acid, L-arginine, fibre, and polyphenols[9]. Habitual nut intake has been associated with reduced risk of cardiovascular disease[10], decreased incidence of the metabolic syndrome[11], and decreased risk of diabetes[12]. Clinical trials have previously explored the effects of nut consumption on markers of inflammation and endothelial function, with a range of effects observed[13-22]. A systematic review and meta-analysis would consolidate and appraise the quality of this body of evidence, providing greater clarity where inconsistencies are observed. Even so, the effort is ongoing. For example, a recently published systematic review did not report significant effects of nut consumption on CRP[23], but did not include results of the large PREDIMED study[24]. It is

also possible to consider FMD as an outcome which this previous review did not consider. The aim of the review reported here was to examine the effect of nut consumption on markers of inflammation and endothelial function (CRP, adiponectin, TNF-α, IL-6, ICAM-1, VCAM-1, FMD) in adults. It was hypothesized that the regular inclusion of nuts in a diet would improve markers of inflammation and endothelial function.

METHODS

This systematic review and meta-analysis followed the requirements of the PRISMA statement[25] (Supplementary material 1). The review was registered in PROSPERO, the international prospective register of systematic reviews (http://www.crd.york.ac.uk/PROSPERO; registration number: CRD42016045424).

Study selection

A systematic search of the databases Medline, PubMed, CINAHL and Cochrane Central Register of Controlled Trials was conducted (all years to 13 January 2017). In line with recommendations by Rosen and Suhami[26] both Medline and PubMed were searched to ensure recent studies were detected. Furthermore, where possible, Medical Subject Heading (MeSH) terms as well as free-text search terms were used in the search[26]. Reference lists of eligible articles and relevant reviews were also reviewed for potential studies. An example of the search strategy used is shown in Supplementary material 2. Articles were restricted to those published in English.

To be included in this review, studies were required to meet the following inclusion criteria: 1) randomised controlled trial (including both parallel and cross-over designs) or prospective cohort

design; 2) studies conducted in humans aged 18 years or older; 3) studies assessing the effect of consumption of tree nuts or peanuts on an outcome of interest (CRP, adiponectin, TNF-alpha, IL-6, ICAM-1 VCAM-1, FMD), where the effect of nut consumption could be isolated. The outcomes of interest were selected to cover a suite of biomarkers regularly used in the literature to indicate changes to inflammation and endothelial dysfunction, including in previous meta-analyses exploring the effects of foods and dietary patterns[27 28] ; 4) studies with an intervention duration of three weeks or more (in the case of randomised controlled trials). This minimum duration was selected to ensure included studies reflected sustained changes to inflammation and endothelial function, and to align with similar cut-offs used in other meta-analyses exploring the impact of dietary components on inflammation[27] or the effect of nut consumption on other physiological measures[29 30]. In addition, the following exclusion criteria were applied: 1) studies involving pregnant or breastfeeding women; 2) studies exploring the effects of nut oils or extracts.

Articles were screened based on title and abstract. Full texts were retrieved in the case that an abstract was not available or did not provide sufficient information to draw a conclusion regarding inclusion in the current review. In the case that results from one study were reported in multiple articles, all articles were checked to avoid duplication of study populations in the analysis or overlooking new information on outcomes. Where different information on outcomes were reported across articles, all relevant articles were included in line with the guidelines of the Cochrane Handbook[31]. Where the same outcomes from a single study were reported across multiple articles, decisions relating to article inclusion were based first on the length of follow-up for the outcome, and then by sample size.

Data extraction

The following data were extracted from each study: citation, country, sample size, participant age and body mass index, health status, study design, study duration, nut type, nut dose, details of control arm, and background diet. Mean changes in relevant outcomes were extracted where possible, and in the case that this data was not available, mean final values were retrieved as recommended by the Cochrane Handbook for Systematic Reviews of Interventions[31]. Study authors were contacted for additional details if the published article did not provide sufficient information. Where a study involved more than one intervention group meeting the inclusion criteria, data for the two intervention groups were combined as recommended by the Cochrane Handbook[31]. In the case of the PREDIMED study[24], which included two intervention arms featuring a Mediterranean diet supplemented with either nuts or olive oil, and a low fat control arm, data from the arm receiving the Mediterranean diet with olive oil was treated as the comparator group. This decision was made to ensure outcomes were not confounded by differences in the background diet of the two groups. Where studies reported median rather than mean, medians were used in the meta-analysis, and standard deviation was imputed from interquartile range.

Abstract screening, study inclusion and exclusion, and data extraction were conducted independently by two authors (EN and VG), and any disagreements were resolved via consensus.

Statistical analyses

Review Manager (Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, the Cochrane Collaboration, 2014) was used to conduct random effects meta-analyses to determine the weighted mean differences (WMD) (with 95% confidence intervals) in change or final mean values for each outcome. In initial analyses, cross-over studies

were treated in the same way as parallel studies by comparing measurements from the intervention periods with the control periods via a paired analysis, as the most conservative approach to managing cross-over studies[31]. In order to explore whether this approach affected the final result by under-weighting these studies, paired analyses of cross-over studies using correlation coefficients of 0.25, 0.5, and 0.75 were conducted as sensitivity analyses.

The proportion of total variation attributable to between-study heterogeneity was estimated using the I^2 test statistic[32]. An I^2 value of 75% or greater was deemed to indicate a high level of inconsistency, based on the recommendations by Higgins et al.[32]. I^2 values were generated for each analysis, including sub-group analyses (outlined below). For outcomes with ten or more strata, funnel plots were generated to explore small study effects, with Egger's test used to determine the extent of funnel plot asymmetry[33]. Where funnel plot asymmetry was detected, sensitivity analyses were conducted to determine if removing studies eliminated the asymmetry.

In addition to the correlation coefficient sensitivity analyses outlined previously, sensitivity analyses were also conducted to explore the effect of removing studies with imputed standard deviations from analyses, and of removing each individual study in meta-analyses ("leave-one-out" analysis). Pre-specified sub-group analyses were also conducted, based on study duration (less than three months versus more than three months), risk of bias, and nut type. For the purpose of sub-group analyses, studies which compared the effects of two types of nuts to a control[34 35] were classified as 'mixed nut studies'. Post-hoc sub-group analyses were conducted based on health status of participants, whether the energy value of nuts was substituted for other foods, study design (parallel vs cross-over), and nut dose (<50 grams per day versus ≥ 50 grams per day[29]).

Quality assessment

The Cochrane Collaboration Risk of Bias tool[31] was used to determine the risk of bias in included studies. EN and VG separately appraised the risk of bias and disagreements were resolved by discussion until consensus was reached. The quality of the body of evidence was then determined using GRADE[36], which considers study design, risk of bias, inconsistency, indirectness, imprecision, and other considerations such as publication bias. GRADEproGDT software (GRADEpro. [Computer program on www.gradepro.org]. Version April 2015. McMaster University, 2014) was utilized to conduct the quality of evidence appraisal.

RESULTS

Characteristics of included studies

A total of n=5200 articles were identified from the systematic search and review of relevant reference lists. After applying exclusion criteria, n=36 articles describing n=32 studies (n=34 strata in pooled analyses) were included in the systematic review and meta-analysis. The process of study inclusion and exclusion is shown in Figure 1. Data access is available on request.

Characteristics of included studies are shown in Table 1. All included studies were randomised controlled trials. Although prospective cohort study designs were also considered, no cohort studies met the overall inclusion criteria for the review. The most common reason was that the cohort studies did not report on the association between nut consumption and an outcome of interest. Fourteen studies had a parallel design[13 15 16 19 34 37-49], 17 had a cross-over design[14 17 18 20-22 35 50-59]. One study[60] combined a parallel and cross-over design, where participants were initially

randomised to one of two parallel groups (energy adjusted or ad libitum diet). In this study, each group then took part in the cross-over part of the study consisting of a walnut included period and a walnut excluded period. Amongst all studies, duration ranged from four weeks to five years, although 20[14 15 17 18 21 22 34 35 40 41 46 48 51-55 57-59] out of 32 studies (63%) had a duration of less than three months. Studies were conducted in Spain[16 18 20 35 37 42-46 52], the United States[14 17 22 38 40 47 49 51 53 54 57 58 60], Australia[48 50], India[19 39], Canada[55], South Korea[15], China[21], Brazil[41], South Africa[34], Iran[56], New Zealand[13], and Germany[59]. Studies included participants who were healthy[48 51], had risk factors for chronic disease such as overweight or obesity, dyslipidaemia, hypertension, or pre-diabetes[13 17 18 20 35 39-41 46 49 50 52 54 55 57-59], had type 2 diabetes mellitus[14 21 22 47 56], met the criteria for Metabolic Syndrome[15 16 19 34 37], had diagnosed coronary artery disease[53], or included a mixture of the aforementioned conditions[38 42-45 60]. Included studies examined the effects of consumption of a range of tree nuts including walnuts[17 18 22 38 49 51 52 54 59 60], almonds[21 40 47 53 55 57], pistachios[14 19 20 39 56 58], hazelnuts[13 46], mixed nuts[15 16 37 42-45], and Brazil nuts[48], as well as peanuts[41 50]. In addition, two studies included multiple intervention arms, featuring a different type of nut in each(walnuts and cashews[34], and walnuts and almonds[35]), compared to a control arm. Nuts were consumed in either prescribed doses, ranging from approximately 18[48] to 85 grams per day[53], or were designed to provide a set proportion of dietary energy, so the amount would vary for individuals[14 18 19 21 34 49 57 58]. Background diets consisted of either participant's habitual diet, or a prescribed diet aligned with healthy lifestyles such as the NCEP Step I or II diet, a Mediterranean-style diet, the Therapeutic Lifestyle Changes diet or another prudent style diet in line with dietary guidelines. Six studies provided all or the majority of foods under controlled feeding conditions[14 21 34 54 57 58]. Twenty-two studies[14 17-22 34 35 38 39 41-46 49 52-55 57-59] prescribed diets accounting for the energy value of the nuts, either quantitatively through dietary

modelling (including the energy value of the nuts within the total energy value of the diet) or qualitatively by encouraging participants to substitute nuts for items with similar energy values. One study[60] included an intervention group where participants were advised on food substitutions to account for the energy value of the provided nuts, and another intervention group where energy intake was not prescribed (ad libitum food consumption). During the control diets or periods, participants typically consumed a similar diet but without nuts, although some studies included control diets with a specific product substituted for the nuts, such as eggs[51], olive oil[35 42-45], muffins[55], and chocolate[40], amongst others. Only two studies[41 49] stated they prescribed a set energy restriction for both intervention and control groups; all other studies utilised isocaloric diets for weight maintenance or ad libitum diets. No studies reported a significant difference in weight loss between the intervention and control groups.

Table 1: Characteristics of included randomised controlled trials examining the effect of nut consumption on inflammatory biomarkers and endothelial function

Citation and country	Sample size (for analysis)	Mean age, years	Mean BMI, kg/m^2	Population	Design	Study duration, weeks	Nut type	Nut dose	Comparison group details	Background diet	Outcome of interest
Barbour et al. (2015)[50], Australia	61 (M: 29, F: 32)	65 ± 7	31 ± 4	Overweight	X	12	Peanut (high oleic)	M: 84g, 6 x week F: 56g, 6 x week	No nuts	Habitual diet	CRP (mg/L)
Burns-Whitmore et al. (2014)[51], United States	20 (M: 4, F: 16)	38 ± 3	23 ± 1	Healthy	X	8	Walnut	28.4g, 6 x week	Standard egg, 6x week*	Habitual diet	CRP (ng/mL)‡‡‡, TNF-α (pg/mL), IL-6 (pg/mL), ICAM-1 (ng/mL)
Canales et al. (2011)[52], Spain	22 (M: 12, F: 10)	54.8 (SEM: 2.0)	29.6 (SEM: 0.7)	Overweight with at least one risk factor for CVD	X	5	Walnut	150g/week walnut paste integrated into steaks and sausages	Low-fat steaks and sausages	Habitual diet with substituted meat products	ICAM-1 (µg/L)‡‡‡, VCAM-1 (µg/L)‡‡‡
Casas-Agustench et al. (2011)[16], Lopez-Uriarte et al. (2010)[37], Spain	50 (M: 28, F: 22)	*I:* 52.9 ± 8.4 *C:* 50.6 ± 8.4	*I:* 31.6 ± 2.8 *C:* 30.0 ± 3.3	MetS	P	12	Mixed nuts (walnut, almond, hazelnut)	30g/day (15g walnuts, 7.5g almonds, 7.5g hazelnuts)	No nuts	American Heart Association dietary guidelines	CRP (mg/L), adiponectin (ng/mL)‡‡‡, IL-6 (ng/L)‡‡‡, ICAM-1 (µg/L)‡‡‡, VCAM-1 (µg/L)‡‡‡
Chen et al. (2015)[53], United States	45 (M: 18, F: 27)	61.8 ± 8.6	30.2 ± 5.1	CAD	X	6	Almond	85g/day	No nuts	NCEP Step 1 diet (isocaloric)	CRP (mg/L), TNF-α (pg/mL), IL-6 (pg/mL),

											VCAM-1 (ng/mL), FMD (%)
Chiang et al. (2012)[54], United States	25 (M: 14, F: 11)	33 (range 23 - 65)	24.8 (range: 18.7 - 36.6)	Normal to HL	X	4	Walnut	42.5g per 10.1MJ (6 x week)	No nuts or fatty fish*	American Dietary Guidelines (isocaloric)	CRP (mg/L)***, TNF-α (pg/mL), IL-6 (pg/mL), ICAM-1 (ng/mL)
Damasceno et al. (2011)[35], Spain	18 (M: 9, F: 9)	56 ± 13	25.7 ± 2.3	HC	X	4	1.Walnut 2. Almond	1. 40 - 65g/day walnuts 2. 50 - 75g/day almonds§ §§	35 – 50g/day virgin olive oil	Mediterranean -style diet (isocaloric)	CRP (mg/L), ICAM-1 (ng/mL), VCAM-1 (ng/mL)
Djousse et al. (2016)[38], United States	26 (M: 10, F: 16)**	*I:* 60.8 ± 11.3 *C:* 68.8 ± 10.9	*I:* 29.6 ± 5.2 *C:* 33.5 ± 8.7	CAD or T2DM	P	12	Walnut	28g/day	No nuts	Habitual diet with walnuts substituted for equivalent kJ items	Adiponectin (µg/mL)
Gulati et al. (2014)[19], India	68 (M: 37, F: 31)	42.5 ± 8.2	30.9 ± 7.5	MetS	P	24	Pistachio	20% of total energy•••	Dietary guidelines for Asian Indians	Dietary guidelines for Asian Indians, with pistachios substituted for diet components	CRP (mg/L)***, adiponectin (µg/mL)***, TNF-α (pg/mL)
Hernandez-Alonso et al. (2014)[20], Spain	54 (M: 29, F: 25)	55 (95% CI: 53.4, 56.8)	28.9 (95% CI: 28.2, 29.6)	Pre-diabetic	X	16	Pistachio	57g/day	Intake of fatty foods adjusted to account for energy from pistachios	Isocaloric diet	Adiponectin (µg/mL)***, IL-6 (pg/mL)
Hu et al. (2016)[48], Australia	21 (M, F)‡‡	*I*: 62.4 ± 8.8 *C*: 66.5 ± 6.9	*I*: 82.2 ± 10.8 *C*: 83.9 ± 22.4§§	Healthy	P	6	Brazil nut (plus green tea extract)	18g/day¶¶	Green tea extract, no nuts	Habitual diet	CRP (mg/L)
Jenkins et al. (2002)[55], Canada	27 (M: 15, F: 12)	64 ± 9	25.7 ± 3.0	HL	X	4	Almond	73 ± 3 g/day¶¶	147 ± 6 g/day muffins¶¶,*	NCEP Step 2 diet (isocaloric)	CRP (mg/L)
Kasliwal et al.	56 (M: 46,	39.3 ±	*I:* 26.1 ±	DL	P	12	Pistachio	40g/day	No nuts	Therapeutic	CRP

(2015)[39], India	F:10) (randomised) 42 (completed)	8.1††	2.9†† *C:* 27.8 ± 4.7††					shelled		Lifestyle Change diet	(mg/L), FMD (%)
Katz et al. (2012)[17], United States	46 (M: 18, F: 28)	57.4 ± 11.9	33.2 ± 4.4	Overweight plus risk factors for MetS	X	8	Walnut	56g/day	No nuts	Ad libitum, participants advised to substitute walnuts for other foods	FMD (%)
Kurlansky and Stote (2006)[40], United States	47 (F)	*Almond:* 41.8 ± 11.7 *Almond + chocolate:* 46.2 ± 7.8 *Chocolate :* 36.5 ± 11.9 *C:* 51.3 ± 6.3	*Almond:* 25.3 ± 3.5 *Almond + chocolate:* 27.2 ± 4.2 *Chocolate:* 23.9 ± 3.3 *C:* 26.1 ± 4.1	Healthy, including HC	P	6	Almond	1. 60g/day 2. 60g almonds/ day + 41g dark chocolate/ day	1. 41g dark chocolate/day 2. self-selected diet	Therapeutic Lifestyle Change diet (isocaloric)	CRP (mg/L), ICAM-1 (ng/mL), VCAM-1 (ng/mL)
Lee et al. (2014)[15], South Korea	60 (M, F)‡‡	ages 35 - 65 eligible for study	*I:* 27.19 ± 2.11 *C:* 26.96 ± 2.16	MetS	P	6	Mixed nuts (walnut, pine nut, peanut)	30g mixed nuts/day (15g walnuts, 7.5g pine nuts, 7.5g peanuts)	Prudent diet	Prudent diet (isocaloric)	CRP (mg/L), adiponectin (µg/mL), IL-6 (pg/mL), ICAM-1 (ng/mL), VCAM-1 (ng/mL)
Liu et al. (2013)[21], China	20 (M: 9, F: 11)	58 ± 2	26.0 ± 0.7	T2DM and HL	X	4	Almond	56g/day¶¶ (20% energy)	NCEP Step II diet	NCEP Step II diet (isocaloric diet)	CRP (mg/L), TNF-α (ng/L)‡‡‡, IL-6 (ng/L)‡‡‡, ICAM-1 (µg/L)‡‡‡, VCAM-1 (µg/L)‡‡‡
Ma et al. (2010)[22],	24 (M: 10, F: 14)	58.1 ± 9.2	32.5 ± 5.0	T2DM	X	8	Walnut	56g/day	No nuts	Ad libitum, participants	FMD (%)

United States										advised to substitute walnuts for other foods	
Moreira Alves et al. (2014)[41], Brazil	65 (M)	*High oleic peanuts:* 27.2 ± 6.1 *Peanuts:* 27.6 ± 1.5 *C:* 27.1 ± 1.6	29.8 ± 2.3	Overweight	P	4	Peanut (high oleic and con-ventional)	1. 56g/day high oleic peanuts 2. 56g/day conventio nal peanuts	No peanuts	Hypocaloric diet (250 kcal/day deficit)	CRP (mg/L)***, TNF-α (pg/mL)
Mukuddem-Petersen et al. (2007)[34], South Africa	64 (M: 29, F: 35)	45 ± 10	*Walnut:* 36 (95% CI: 33.3 - 38.7) *Cashew:* 34.4 (95% CI: 32.3 - 36.6) *C:* 35.1 (95% CI: 32.8 - 37.4)	MetS	P	8	1. Walnut 2. Cashew	1. 20% energy from walnuts 2. 20% energy from cashews§ §§	No nuts	Controlled feeding protocol (isocaloric)	CRP (mg/L)
Njike et al. (2015)[60], United States	112 (M: 31, F: 81)	*Ad libitum:* 56.5 ± 11.7 *Energy adjusted:* 53.3 ± 11.1	*Ad libitum:* 30.0 ± 4.0: *Energy adjusted:* 30.2 ± 4.1	Overweight, pre-diabetic or MetS	X••	24	Walnut	56g/day	No nuts	1. Ad libitum diet 2. Isocaloric diet (energy adjusted for walnuts)	FMD (%)
Parham et al. (2014)[56], Iran	44 (M: 11, F: 33)	*Interventi on first:* 53 ± 10 *Control first:* 50 ± 11	*Intervention first:* 32.16 ± 6.58 *Control first:* 30.24 ± 4.03	T2DM	X	12	Pistachio	50g/day	No pistachios	Ad libitum	CRP (mg/dL)‡‡‡
PREDIMED (Casas et al., 2014[42], Casas et al., 2016[43], Lasa et al., 2014[44], Urpi-Sarda et al., 2012[45]), Spain	353 (M: 172, F: 181)‡ 124 (M: 45, F: 79)• 110 (M: 55, F: 55)§ 108 (M: 54, F: 54)¶	Range: 55 – 80 (M), 60 – 80 (F)	29.4 ± 3.4‡	T2DM and/or CHD risk factors	P	52 ‡,•,§ 260 (5 years)¶	Mixed nuts (walnut, almond, hazelnut)	30g/day (15g walnuts, 7.5g hazelnuts, 7.5g almonds)	1L olive oil per week†	Mediterranean diet	CRP (mg/L) †††, adiponectin (µg/mL), TNF-α (pg/mL), IL-6 (pg/mL), ICAM-1 (µg/L)‡‡‡,

											VCAM-1 (ng/mL)
Rajaram et al. (2010)[57], United States	25 (M: 14, F: 11)	41 (SEM: 13)	71 (SEM: 2.7)§§	Healthy (including overweight) to HC	X	4	Almond	1. 10% energy 2. 20% energy§§§	No nuts	Cholesterol lowering diet (isocaloric)	CRP (mg/L), IL-6 (ng/L)‡‡‡
Rock et al. (2016)[49], United States	126 (F)	50 (range: 22 - 72)††	33.5 (range: 27 - 40)††	Overweight	P	52	Walnut	42g/day¶¶ (18% energy)	1. higher fat (35% energy) lower CHO (45% energy) diet, no nuts*	Hypocaloric diet (500 - 1000 kcal/day deficit)	CRP (ug/mL)‡‡‡, IL-6 (pg/mL)
Ros et al. (2004)[18], Spain	20 (M: 8, F: 12)	55 (range: 26 - 75)	70.6 ± 10.3§§	HC	X	4	Walnut	40 – 65g/day (~18% energy) §§§	No nuts	cholesterol lowering Mediterranean diet (isocaloric)	CRP (mg/L)***, ICAM-1 (µg/L)‡‡‡, VCAM-1 (µg/L)‡‡‡, FMD (%)
Sauder et al. (2015)[14], United States	30 (M: 15, F: 15)	56.1 ± 7.8	31.2 ± 3.1	T2DM	X	4	Pistachio	20% total energy§§§	Therapeutic Lifestyle Changes diet	Therapeutic Lifestyle Changes diet (isocaloric)	CRP (mg/L), ICAM-1 (ng/mL), VCAM-1 (ng/mL), FMD (%)
Sola et al. (2012)[46], Spain	56 (M: 23, F: 33)	*I:* 56.79 ± 10.46 *C:* 49.79 ± 9.53	*I:* 27.30 ± 3.01 *C:* 28.31 ± 3.25	Pre-HT or HT with at least one risk factor for CVD	P	4	Hazelnut	30g/day (in cocoa cream product)	Cocoa cream product*	Low saturated fat diet (isocaloric)	CRP (mg/L), IL-6 (pg/mL), ICAM-1 (ng/mL), VCAM-1 (ng/mL)
Sweazea et al. (2014)[47], United States	21 (M: 9, F: 12)	*I:* 57.8 ± 5.6 *C:* 54.7 ± 8.9	*I:* 37.2 ± 7.8 *C:* 33.5 ± 8.8	T2DM	P	12	Almond	43g (5-7 x week)	≤ 2 servings non-trial nuts/week	Habitual diet	CRP (mg/L), TNF-α (pg/mL), IL-6 (pg/mL)
Tey et al. (2014)[13], New Zealand	107 (M: 46, F: 61)	42.5 ± 12.4	30.6 ± 5.1	Overweight	P	12	Hazelnut	1. 30g/day 2. 60g/day	No nuts	Habitual diet	CRP (mg/L), IL-6 (pg/mL), ICAM-1 (µg/L)‡‡‡, VCAM-1

											(µg/L)‡‡‡
West et al. (2012)[58], United States	28 (M: 10, F: 18)	48 (SEM: 1.5)	26.8 (SEM: 0.7)	HL	X	4	Pistachio	1. 10% energy 2. 20% energy§§§	NCEP Step 1 diet	Isocaloric diet	FMD (%)
Wu et al. (2014)[59], Germany	40 (M: 10, F: 30)	60 ± 1	24.9 ± 0.6	Healthy (including overweight)	X	8	Walnut	43g/day	No nuts	Western diet with walnuts substituted for saturated fat (isocaloric)	CRP (mg/dL)‡‡‡, adiponectin (µg/mL)***, ICAM-1 (ng/mL), VCAM-1 (ng/mL)

*Study included other intervention group which was not relevant to this review, therefore this group was not included in this analysis
†Treated as comparison group for this analysis
‡ICAM [45]
•Adiponectin [44]
§VCAM-1 [42]
¶CRP, IL-6, TNF-α [43]
**Gender breakdown estimated from % males reported in paper
††Characteristics reported for randomised participants
‡‡Gender breakdown for analysed participants not available
••Participants were randomised to one of two parallel groups (ad libitum or calorie adjusted). Within each group participants completed a 'walnut included' and 'walnut excluded' period in a cross-over design
§§ Body weight (kg) is reported when BMI was not available
¶¶ Mean intake
•••Dose based on reference individual listed in Gulati et al. [19]
§§§Gram weight for dose sub-analysis based on mid-point of range of doses used
***Units confirmed with study authors
††† Units based on primary publication[61]
‡‡‡Unit reported in study, converted to consistent unit for analysis
Abbreviations: BMI: body mass index; CAD: coronary artery disease; CHD: coronary heart disease; CI: confidence intervals; CVD: cardiovascular disease; DL: dyslipidaemia; F: female; HL: hyperlipidaemia; HT: hypertension; M: male; MetS: metabolic syndrome; NCEP: National Cholesterol Education Program; P: parallel; SEM: standard error of mean; T2DM: type 2 diabetes mellitus; X: cross-over

Effect of nut consumption on study outcomes

FMD

A total of nine strata from eight studies[14 17 18 22 39 53 58 60] explored the effect of nut consumption on FMD. Of the nine strata, five explored the effect of walnut consumption on FMD[17 18 22 60], and six had a duration of less than three months[14 17 18 22 53 58]. The meta- analysis showed that nut consumption was associated with a significant increase in FMD (Figure 2 and Table 2). Sensitivity analyses indicated that excluding any one study did not substantially alter the effect (data not shown). The effect estimate was also similar after using different correlation coefficients (CC: 0.5, Supplementary material 3; CC: 0.25 and 0.75, data not shown). No significant differences were found for sub-group analyses (Supplementary material 4) although it was noted that when sub-group comparisons were made according to nut type, only the walnut sub-group found significant improvements in FMD.

CRP

A total of 26 strata from 25 studies[13-16 18 19 21 34 35 39-41 43 46-51 53-57 59] explored the effect of nut consumption on CRP. Almonds were the most common nut type used in these analyses (seven strata[21 40 47 53 55 57]), followed by walnuts[18 49 51 54 59] and mixtures of more than one nut type[15 16 34 35 43] (each used in five strata). A total of 17 strata from 16 studies had a duration of less than three months[14 15 18 21 34 35 40 41 46 48 51 53-55 57 59]. When all studies were included in the meta-analysis, nut consumption resulted in non-significant differences in CRP (Figure 3 and Table 2). The overall effect was relatively unchanged when studies with imputed standard deviations were removed from the analysis (Table 2). Sensitivity analyses identified two studies[15 51] that contributed substantially to the pooled result, as when they were excluded from the meta-

analysis, the reductions in CRP were significant (Supplementary material 5). In addition, the use of different correlation coefficients did not change the overall effect found (CC: 0.5, Supplementary material 3; CC: 0.25 and 0.75, data not shown). Sub-group analyses indicated that statistically significant differences were found between studies which included the energy value of nuts in the prescribed diet compared to those that did not (Supplementary material 4). An effect estimate of -0.23 mg/L [-0.44, -0.01] was found for studies in which diets incorporated the energy value of nuts, whilst an effect estimate of -0.00 mg/L [-0.06, 0.05]) was found for studies which did not ($Chi^2 = 3.99$, df = 1 (P = 0.05), $I^2 = 74.9\%$). When studies were grouped according to nut dose, an effect estimate of -0.00 mg/L [0.00, 0.00] was found for studies which included less than 50 grams of nuts/day, whilst an effect estimate of -0.34 mg/L [-0.63, -0.06]) was found when 50 grams or more were used ($Chi^2 = 5.74$, df = 1 (P = 0.02), $I^2 = 82.6\%$). Borderline significant differences (p=0.05) were found when studies with a parallel design were compared to cross-over studies. However, when either of the studies identified in the sensitivity analysis[51,15] were excluded, these sub-group analyses no longer produced significant results (data not shown).

Adiponectin, TNF-α, IL-6, ICAM-1, VCAM-1

The meta- analysis showed that consumption of nuts did not result in significant differences in adiponectin, TNF-α, IL-6, ICAM-1, or VCAM-1 (Table 2 and Supplementary material 6). In the case that pooled analyses featured studies with imputed standard deviations (IL-6, ICAM-1, VCAM-1), excluding these studies did not substantially change the effect estimates (Table 2). Sensitivity analyses indicated that excluding any one study did not substantially alter the effect (data not shown). Overall effects also did not change when different correlation coefficients were used for cross-over studies (CC: 0.5, Supplementary material 3; CC: 0.25 and 0.75, data not

shown). No significant differences between sub-groups were observed (Supplementary material 4).

Table 2: Differences in FMD, CRP, adiponectin, TNF-α, IL-6, ICAM-1, and VCAM-1following nut consumption, compared to control.

Outcome	Analysis description	Number of studies	Number of strata	Number of participants	Effect estimate		Inconsistency (I^2)
FMD (%)	**All studies‡**	8	9	652	0.79% [0.35, 1.23], P<0.001	-0.40% [-1.72, 0.92] - 2.36% [-1.71, 6.43]	0%
CRP (mg/L)	**All studies**	25	26	1578	-0.01mg/L [-0.06, 0.03], P = 0.59†	-5.53mg/L [-11.96, 0.90] - 0.60mg/L [-2.44, 3.64]	20%
	Imputed SD excluded*	19	20	1244	-0.01mg/L [-0.06, 0.04], P = 0.71	-5.53mg/L [-11.96, 0.90] - 0.60mg/L [-2.44, 3.64]	26%
Total adiponectin (µg/mL)	**All studies‡**	7	7	506	0.29 µg/mL [-0.63, 1.21], P = 0.53	-9.80µg/mL [-23.99, 4.39] - 10.60µg/mL [6.39, 14.81]	79%
TNF-α (pg/mL)	**All studies‡**	8	8	482	-0.05 pg/mL [-0.13, 0.02], P = 0.17	-3.70pg/mL [-6.93, -0.47] - 0.70pg/mL [-0.41, 1.81]	2%

IL-6 (pg/mL)	**All studies**	13	13	906	-0.02 pg/mL [-0.12, 0.08], P = 0.65,	-1.55pg/mL [-2.80, -0.30] - 0.46pg/mL [-0.22, 1.14]	10%
	Imputed SD excluded	11	11	800	-0.09 pg/mL [-0.23, 0.05], P = 0.19	-0.50pg/mL [-1.62, 0.62] - 0.46pg/mL [-0.22, 1.14]	0%
ICAM-1 (ng/mL)	**All studies**	14	15	1047	0.68 ng/mL [-0.53, 1.89], P = 0.27	-80.63ng/mL [-209.62, 48.36] - 16.76ng/mL [1.44, 32.08]	0%
	Imputed SD excluded	13	14	1011	0.68 ng/mL [-0.53, 1.89], P = 0.27	-80.63ng/mL [-209.62, 48.36] - 16.76ng/mL [1.44, 32.08]	0%
VCAM-1 (ng/mL)	**All studies**	13	14	804	2.83 ng/mL [-8.85, 14.51], P = 0.63	-99.72ng/mL [-316.35, 116.91] - 62.00ng/mL [-80.23, 204.23]	0%
	Imputed SD excluded	12	13	768	2.43 ng/mL [-9.29, 14.15], P = 0.68	-99.72ng/mL [-316.35, 116.91] - 46.34ng/mL [-	0%

						22.06, 114.75]	

*Sensitivity analysis where studies with an imputed standard deviation were excluded

†Sensitivity analyses indicated that exclusion of either of two studies[15 51] resulted in an effect estimate of -0.22 [-0.40, -0.04].

‡No studies reporting FMD, adiponectin or TNF-α, required imputation of standard deviation

Small study effects

Funnel plots were generated for outcomes with ten or more strata (CRP, IL-6, ICAM-1, and VCAM-1) (Supplementary material 7). Egger's test indicated asymmetry in funnel plots for CRP (bias = -0.69 [95% CI = -1.07 to -0.31], P = 0.001) and IL-6 (bias = -0.80 [95% CI = -1.45 to -0.16], P = 0.02), suggesting the presence of small study effects which may have been attributable to publication bias. Sensitivity analyses attempting to eliminate studies which appeared to be responsible for the small study effects did not alleviate the asymmetry found (data not shown). Funnel plot asymmetry was not detected for ICAM-1 or VCAM-1 (data not shown).

Risk of bias and quality of the body of evidence

The risk of bias was determined for each strata using the Cochrane Risk of Bias Tool and the results of the assessment are shown in Figure 4 and Supplementary materials 8 and 9. The quality of the evidence was 'high' for FMD, ICAM-1, and VCAM-1. The quality was downgraded to 'moderate' for TNF-α due to risk of bias, and to 'low' for CRP and IL-6 due to both risk of bias and the possibility of publication bias. The quality of the evidence for adiponectin was downgraded to 'very low' due to risk of bias, inconsistency, and imprecision (Supplementary material 10).

DISCUSSION

The results of this systematic review and meta-analysis suggested favourable effects of nut consumption on FMD, a measure of endothelial function. These findings align with a review conducted in 2011 by the European Food Safety Authority (EFSA), which explored the effects of walnut consumption on endothelium-dependent vasodilation [62]. A meta-analysis was not part

of the EFSA report[62], but the present study provides a meta-analysis that includes more recently published research[17 60]. It also includes studies investigating other types of nuts[14 39 53 58]. Sub-group analyses found significant improvements in FMD only in those studies using walnuts, consistent with the EFSA report which only examined walnut consumption, although the test for sub-group differences in the present study did not reach statistical significance. This may have resulted from the small number of studies available for assessing FMD. Having few studies may have also played a role in the lack of significant effects observed in other FMD sub-group analyses. These include studies in participants with type 2 diabetes, or studies lasting longer than three months. Further research is therefore required in this area.

Despite the small sample size, the findings of this review relating to FMD are of value due to the known associations between FMD and future cardiovascular events. A meta-analysis of cohort studies found a significant reduction in risk of cardiovascular events per 1% increase in FMD (RR: 0.872 [95% CI: 0.832 – 0.914])[8]. In comparison, the present study found an effect estimate of 0.79% for nut consumption compared to controls, suggesting these results are likely to be of clinical relevance to future cardiovascular risk. There are a number of mechanisms by which nuts, and walnuts in particular, could improve FMD. FMD is a measure of endothelial dysfunction[63], a condition characterised by reduced availability of the vasodilator nitric oxide (NO)[64]. Nuts contain high levels of L-arginine[65], an amino acid which acts as a precursor to NO[66]. Walnuts in particular are rich in alpha-linolenic acid, a polyunsaturated fatty acid that has been suggested to increase membrane fluidity, thus also increasing nitric oxide synthesis and release[67]. The antioxidant content of nuts may also play a role in the improvements in endothelial function observed[9].

Our finding of no significant effects on inflammatory biomarkers CRP, TNF-α, IL-6, ICAM-1, VCAM-1, or the anti-inflammatory biomarker adiponectin reflects the body of evidence available at this time. There may be effects with CRP but characteristics of the study sample or design of the dietary intervention may influence the ability to detect these effects. Sensitivity analyses indicated that results may have been disproportionally influenced by a small number of studies. Exclusion of either one of two studies[15 51] resulted in the meta-analysis yielding significant reductions in CRP following nut intake, suggesting these two studies were responsible for the results found. This appears to be the result of low reported CRP values and correspondingly small standard errors, resulting in these studies receiving substantially higher weighting than other studies in the pooled analysis. The study sample may in part explain these findings, as the study by Burns-Whitmore et al.[51] was conducted in healthy lacto-ovo vegetarians. Consumption of a plant-based diet has been associated with decreased inflammation[68]. In contrast, Lee et al.[15] explored the effect of nut consumption in individuals with Metabolic Syndrome, which is typically associated with elevated CRP levels[69]. Reported units were confirmed with study authors.

The findings of this review may also have been influenced by the design of the dietary interventions included. Sub-group analyses found significant reductions in CRP when studies incorporated 50 grams or more of nuts per day. This finding aligns with previous research suggesting a dose-response effect of nut intake on other outcomes such as cholesterol[70]. However, these findings should be interpreted with caution, as several studies[14 18 19 21 34 49 57 58] incorporated nuts as a proportion of total energy, resulting in substantial variation between individuals in the dose consumed. Furthermore, whether the energy value of nuts was adjusted for in the total diet may have influenced results. Sub-group analyses suggested significant effects

on CRP were only found when the energy provided by nuts was accounted for either by dietary modelling or advice to substitute other foods for nuts. This aligns with a previous review by our group which highlighted the importance of considering total energy intake in trials examining the effect of vegetable intake on weight loss[71]. There is also evidence to suggest markers of inflammation such as CRP may be reduced following periods of energy restriction[72], highlighting the importance of considering total energy intake when exploring the effects of individual foods. The design of the control arm may have also impacted on results, as several studies[35 42-45] compared intake of nuts to a control intervention which also had the potential to influence inflammation and endothelial function, for example olive oil[27]. The potential impact of control groups on underestimating intervention effects has previously been highlighted in the weight loss literature[73]. Trials aiming to explore the influence of specific foods on health outcomes must carefully consider the design of the dietary intervention and control arms, and aim to avoid increases in total energy intake which could skew results.

The heterogeneity in study design elements, particularly related to dietary intervention, may explain why reviews exploring the effects of nut consumption on inflammation have found varying results. Although including fewer studies than in our review, a recently published review by Mazidi et al.[23] also found non-significant differences in inflammatory biomarkers (CRP, IL-6, adiponectin, ICAM-1, and VCAM-1), although in contrast to our review they observed a small increase in CRP levels. The review by Mazidi et al.[23] appeared to have broader eligibility criteria which also included post-prandial studies and those exploring the effects of soy consumption. In another review Barbour et al.[74] reported significant reductions in CRP following nut consumption. It should be noted however, that Barbour et al.[74] included studies where nut consumption was encouraged as part of a suite of favourable dietary changes not matched in

control groups, meaning the effect of the nuts themselves could not be isolated. In these circumstances it may not be possible to show whether effects observed were the result of increases in nut intake, or the wider dietary changes occurring. We avoided this problem by excluding studies with a portfolio of dietary changes not matched in the control group, or by treating a comparable intervention group as the "control" (or comparator), as in the case of the PREDIMED study[24]. Nevertheless, nuts appear in healthy dietary patterns and we have previously shown that consumption of a healthy dietary pattern (many of which include habitual nut intake) results in significant reductions in CRP[75].

It should be noted that while the current analysis found favourable effects of nut consumption on a marker of endothelial dysfunction, the lack of evidence for effects on cell adhesion molecules VCAM-1 and ICAM-1 suggests changes in endothelial cell activation may not have occurred. Given that the inflammatory cytokines which characteristically induce endothelial cell activation (for example TNF-α and IL-6)[64] also appeared unchanged, the lack of difference found for ICAM-1 and VCAM-1 is perhaps not surprising. More research on this cluster of molecules will be informative.

This review had a number of strengths. It used a systematic methodology following current guidelines for systematic reviews, including prospective registration, and used the Cochrane Risk of Bias tool and GRADE method to evaluate the quality of evidence. We considered a range of biomarkers associated with inflammation and endothelial function, including the anti-inflammatory adipocyte adiponectin. These biomarkers were selected to reflect changes in disease progression and amelioration, in order to explore mechanisms responsible for the favourable effects of nut consumption on cardiovascular disease[10] and other chronic conditions[11] [12]. However we fully acknowledge that the measures explored here are not interchangeable with

disease endpoints such as mortality and morbidity. The size of the evidence base, including the small number of participants available for analyses of individual biomarkers, is a limitation, particularly with respect to generalisability and strength of the evidence. Furthermore, although we were unable to explore the distribution of the published data included in this meta-analysis, the fact that several studies reported median values rather than means suggests some of the data may have been skewed, which may have impacted upon our analyses.

The heterogeneity of the evidence base included can be also considered a limitation of this review. Variation existed as a result of participant health status, nut type and dose, and study duration, although these factors were explored in sub-group analyses. Statistically significant sub-group differences were found only for CRP when studies were grouped according to whether they incorporated the energy value of nuts into the diet, and based on nut dose (<50 grams/day versus ≥50 grams/day). However due to the small number of studies, it is possible that other sub-group differences may have been found if the sample size was larger. For example, borderline significant differences (p=0.05) were found between the study designs, with larger reductions in CRP found for cross-over design studies. As the nature of cross-over studies eliminates between-subject variation[76], they may provide superior insights when exploring the impact of dietary interventions on biomarkers such as CRP, however their results may also be impacted by carry-over effects[31]. Given the short or absent wash-out periods of some of the included studies[18 35 50 54 57], the potential impact of carry-over effects cannot be ruled out. Background diets also varied between studies, with some studies prescribing diets based on dietary guidelines, whereas others allowed participants to follow their habitual diet, which may have varied substantially between individuals. Analysis of funnel plots suggested the results for CRP and IL-6 may have been influenced by small study effects (which could indicate publication bias), which resulted in

downgrading the quality of the evidence for these outcomes. Funnel plot asymmetry remained after sensitivity analyses were conducted to remove the studies which appeared to be responsible for these effects. These findings suggest the need for more research in this area, with a particular focus on the registration of study protocols with detailed information on primary and secondary outcomes, to reduce the potential for publication bias.

This systematic review and meta-analysis of the effects of nut consumption on inflammation and endothelial function found evidence for favourable effects on FMD, a measure of endothelial function. Non-significant differences in CRP, adiponectin, TNF-α, IL-6, ICAM-1, VCAM-1 suggest a lack of consistent available evidence for effects of nut consumption on inflammation, although the results for CRP should be interpreted with caution due to the large influence of single studies on the pooled results. The findings of this review provide further insight into the mechanisms by which nut consumption may exert favourable effects on the risk of chronic conditions such as cardiovascular disease. The findings also build on previous research such as the 2011 EFSA report[62] on walnut consumption and endothelial-dependent vasodilation, and reinforce the value of including nuts within a healthy dietary pattern. However, the small evidence base for FMD and the observed lack of consistency in findings relating to inflammation suggest a need for more research in this area, with a particular focus on randomised controlled trials incorporating the energy value of nuts into the total diet. There is also a need for the transparent registration of trial protocols, as well as appropriate dietary controls. These could include healthy dietary patterns (not including nuts), with a greater emphasis on dietary modelling required to ensure nutrient intakes are matched between control and intervention groups, minimising the risk of confounding.

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Figure titles:

Figure 1: PRISMA[25] flow diagram of study selection

Figure 2: Difference in FMD (%) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals.

Figure 3: Difference in C-reactive protein (mg/L) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals.

Figure 4: Risk of bias assessment as proportion of total strata.

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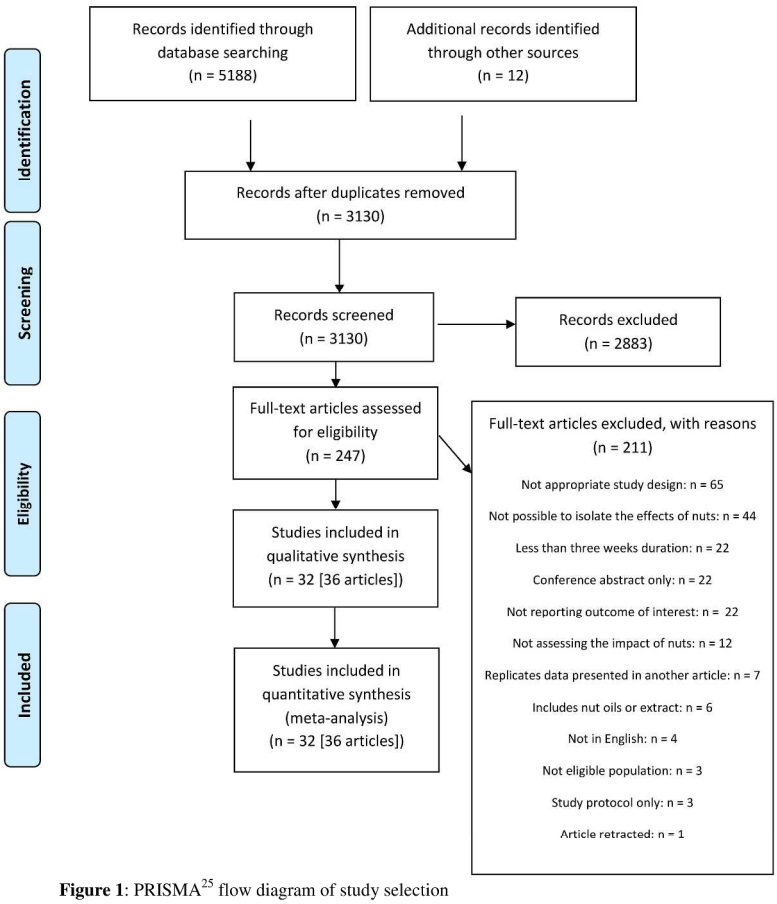

Figure 1: PRISMA[25] flow diagram of study selection

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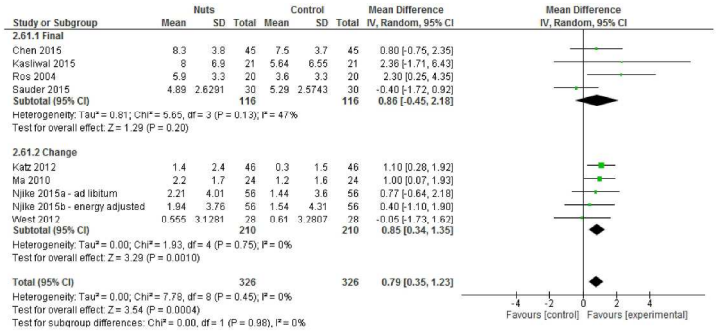

Figure 2: Difference in FMD (%) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals.

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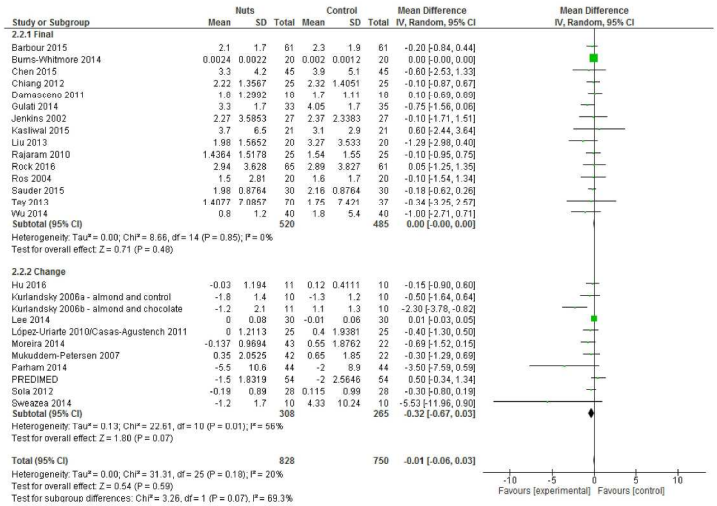

Figure 3: Difference in C-reactive protein (mg/L) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals.

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279x361mm (300 x 300 DPI)

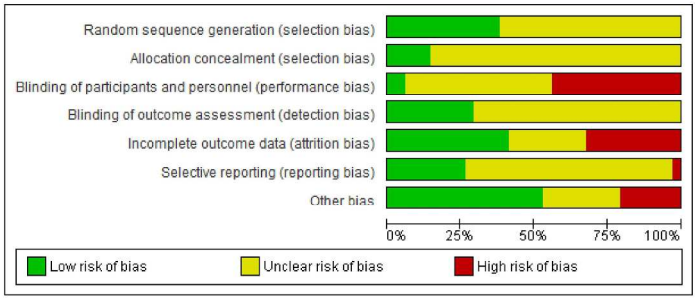

Figure 4: Risk of bias assessment as proportion of total strata.

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279x361mm (300 x 300 DPI)

List of supplementary material

Supplementary material 1: PRISMA checklist (as separate file)

Supplementary material 2: Example search strategy

Supplementary material 3: Differences in CRP, adiponectin, TNF-α, IL-6, ICAM-1, VCAM-1, and FMD following nut consumption, compared to control, using correlation coefficient of 0.5

Supplementary material 4: Results of sub-group analyses

Supplementary material 5: Forest plots of difference in CRP after exclusion of individual studies

Supplementary material 6: Forest plots of differences in biomarkers between nut consumption and control

Supplementary material 7: Funnel plots

Supplementary material 8: Risk of bias assessment summary

Supplementary material 9: Justification for risk of bias judgements

Supplementary material 10: GRADE assessment of the quality of the body of evidence

Supplementary material 2:

Search strategy: PubMed

((((((((((((((((((((("nuts"[MeSH Terms]) OR nut) OR nuts) OR "juglans"[MeSH Terms]) OR walnut*) OR "prunus dulcis"[MeSH Terms]) OR almond*) OR "bertholletia"[MeSH Terms]) OR brazil nut*) OR Amazonia) OR "anacardium"[MeSH Terms]) OR cashew*) OR "corylus"[MeSH Terms]) OR hazelnut*) OR "macadamia"[MeSH Terms]) OR macadamia*) OR "carya"[MeSH Terms]) OR pecan*) OR "pinus"[MeSH Terms]) OR pine nut*) OR "pistacia"[MeSH Terms]) OR pistachio*) OR "arachis"[MeSH Terms]) OR peanut*))

AND

((((((((((((((((((("inflammation"[MeSH Terms]) OR inflammat*) OR endothelial*) OR "adiponectin"[MeSH Terms]) OR adiponectin) OR high molecular weight adiponectin) OR "c reactive protein"[MeSH Terms]) OR c reactive protein) OR c-reactive protein) OR CRP) OR "tumor necrosis factor alpha"[MeSH Terms]) OR tumor necrosis factor*) OR tumour necrosis factor*) OR TNF*) OR "interleukins"[MeSH Terms]) OR interleukin*) OR "cell adhesion molecules"[MeSH Terms]) OR adhesion molecule*) OR flow mediated dilat*) OR flow-mediated dilat*) OR FMD) OR "cytokines"[MeSH Terms]) OR cytokine*)

Supplementary material 3: Differences in CRP, adiponectin, TNF-α, IL-6, ICAM-1, VCAM-1, and FMD following nut consumption, compared to control, using correlation coefficient of 0.5

Outcome	Number of analyses	Number of participants	Effect estimate		Inconsistency (I^2)
CRP (mg/L)	26	1578	-0.03 mg/L [-0.09, 0.03], P = 0.30	-5.53 mg/L [-11.96, 0.90] - 0.60 mg/L [-2.44, 3.64]	33%
Total adiponectin (µg/mL)	7	506	0.15 µg/mL [-0.77, 1.07], P = 0.75	-9.80µg/mL [-23.99, 4.39] - 10.60µg/mL [6.39, 14.81]	81%
TNF-α (pg/mL)	8	482	-0.05 pg/mL [-0.12, 0.02], P = 0.17	-3.70pg/mL [-6.93, -0.47] - 0.70 pg/mL [-0.41, 1.81]	7%
IL-6 (pg/mL)	13	906	-0.06 pg/mL [-0.16, 0.04], P = 0.24	-1.55 pg/mL [-2.80, -0.30] - 0.46 pg/mL [-0.22, 1.14]	28%
ICAM-1 (ng/mL)	15	1047	0.62 ng/mL [-0.24, 1.49], P = 0.16	-80.63ng/mL [-209.62, 48.36] - 16.76ng/mL [1.44, 32.08]	0%
VCAM-1 (ng/mL)	14	804	1.25 ng/mL [-12.09, 14.59], P = 0.85	-99.72ng/mL [-216.35, 116.91] - 62.00ng/mL [-39.40, 163.40]	9%

FMD (%)	9	652	0.74 % [0.27, 1.20], P = 0.002	-0.40% [-1.33, 0.53] - 2.36% [-1.71, 6.43]	46%

Supplementary material 4: Results of sub-group analyses

Table 1: Results of sub-group analyses for CRP

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	17	847	-0.00 mg/L [-0.04, 0.03]	Chi² = 1.02, df = 1 (P = 0.31), I² = 1.9%
	More than three months	9	731	-0.24 mg/L [-0.69, 0.22]	
Risk of bias	Low/unclear	11	588	-0.25 mg/L [-0.53, 0.04]	Chi² = 2.82, df = 1 (P = 0.09), I² = 64.6%
	High	15	990	0.00 mg/L [-0.00, 0.00]	
Nut type	Almond	7	295	-0.79 mg/L [-1.52, -0.06]	Chi² = 10.42, df = 6 (P = 0.11), I² = 42.4%
	Walnut	5	336	0.00 mg/L [-0.00, 0.00]	
	Hazelnut	2	163	-0.31 mg/L [-0.79, 0.18]	
	Mixed nut	5	318	0.01 mg/L [-0.03, 0.05]	
	Peanut	2	187	-0.38 mg/L [-0.89, 0.13]	

	Pistachio	4	258	-0.42 mg/L [-1.03, 0.19]	
	Brazil nut	1	21	-0.15 mg/L [-0.90, 0.60]	
Health status	Healthy	2	61	0.00 mg/L [-0.00, 0.00]	Chi^2 = 10.41, df = 5 (P = 0.06), I^2 = 52.0%
	Chronic disease risk factors	14	869	-0.29 mg/L [-0.54, -0.04]	
	T2DM	4	208	-1.18 mg/L [-2.70, 0.35]	
	MetS	4	242	-0.19 mg/L [-0.55, 0.17]	
	CAD	1	90	-0.60 mg/L [-2.53, 1.33]	
	Combination	1	108	0.50 mg/L [-0.34, 1.34]	
Energy value of nuts included in diet	Adjusted	16	1029	-0.23 mg/L [-0.44, -0.01]	Chi^2 = 3.99, df = 1 (P = 0.05), I^2 = 74.9%
	Not adjusted	10	549	-0.00 mg/L [-0.06, 0.05]	
Study design	Parallel	14	828	-0.29 mg/L [-0.58, 0.00]	Chi^2 = 3.84, df = 1 (P = 0.05), I^2 = 74.0%
	Cross-over	12	750	0.00 mg/L [-0.00, 0.00]	
Nut dose	<50g/day	13	828	0.00 mg/L [-0.00, 0.00]	Chi^2 = 5.74, df = 1 (P = 0.02), I^2 = 82.6%
	≥50g/day	13	750	-0.34 mg/L [-0.63, -0.06]	

Table 2: Results of sub-group analyses for FMD

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	6	386	0.77 % [0.17,1.38]	Chi² = 0.01, df = 1 (P = 0.91), I² = 0%
	More than three months	3	266	0.70 % [-0.29, 1.70]	
Risk of bias	Low/unclear	6	480	0.69 % [0.22, 1.16]	Chi² = 1.32, df = 1 (P = 0.25), I² = 24.2%
	High	3	172	1.43 % [0.25, 2.61]	
Nut type	Almond	1	90	0.80 % [-0.75, 2.35]	Chi² = 3.86, df = 2 (P = 0.15), I² = 48.1%
	Walnut	5	404	1.02 % [0.51, 1.53]	
	Pistachio	3	158	-0.11 % [-1.11, 0.90]	
Health status	Chronic disease risk factors	4	230	1.09 % [0.25, 1.92]	Chi² = 0.97, df = 3 (P = 0.81), I² = 0%
	T2DM	2	108	0.38 % [-0.98, 1.74]	

	CAD	1	90	0.80 % [-0.75, 2.35]	
	Combination	2	224	0.60 % [-0.43, 1.62]	
Energy value of nuts included in diet	Adjusted	8	540	0.77 % [0.27, 1.27]	$Chi^2 = 0.00$, df = 1 (P = 1.00), I^2 = 0%
	Not adjusted	1	112	0.77 % [-0.64, 2.18]	
Study design	Parallel	1	42	2.36 % [-1.71, 6.43]	$Chi^2 = 0.58$, df = 1 (P = 0.45), I^2 = 0%
	Cross-over	8	610	0.77 % [0.32, 1.21]	
Nut dose	<50g/day	1	42	2.36 % [-1.71, 6.43]	$Chi^2 = 0.58$, df = 1 (P = 0.45), I^2 = 0%
	≥50g/day	8	610	0.77 % [0.32, 1.21]	

Table 3: Results of sub-group analyses for adiponectin

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	2	130	-0.60 µg/mL [-2.48, 1.28]	Chi² = 1.03, df = 1 (P = 0.31), I² = 3.3%
	More than three months	5	376	1.71 µg/mL [-2.33, 5.75]	
Risk of bias	Low/unclear	3	234	-0.00 µg/mL [-0.00, 0.00]	Chi² = 0.45, df = 1 (P = 0.50), I² = 0%
	High	4	272	1.91 µg/mL [-3.70, 7.53]	
Nut type	Walnut	2	96	-0.52 µg/mL [-3.78, 2.75]	Chi² = 0.57, df = 2 (P = 0.75), I² = 0%
	Mixed nut	3	234	-0.00 µg/mL [-0.00, 0.00]	
	Pistachio	2	176	4.49 µg/mL [-8.30, 17.28]	
Health status	Chronic disease risk factors	2	178	-2.33 µg/mL [-5.28, 0.63]	Chi² = 3.42, df = 2 (P = 0.18), I² = 41.5%
	MetS	3	178	0.53 µg/mL [-0.49, 1.55]	

	Combination	2	150	-2.05 μg/mL [-11.64, 7.54]	
Energy value of nuts included in diet	Adjusted	5	396	0.80 μg/mL [-4.62, 6.22]	Chi² = 0.08, df = 1 (P = 0.77), I² = 0%
	Not adjusted	2	110	-0.00 μg/mL [-0.00, 0.00]	
Study design	Parallel	5	328	0.53 μg/mL [-0.43, 1.49]	Chi² = 3.24, df = 1 (P = 0.07), I² = 69.2%
	Cross-over	2	178	-2.33 μg/mL [-5.28, 0.63]	
Nut dose	<50g/day	6	398	0.34 μg/mL [-0.60, 1.28]	Chi² = 0.49, df = 1 (P = 0.48), I² = 0%
	≥50g/day	1	108	-2.48 μg/mL [-10.31, 5.35]	

Table 4: Results of sub-group analyses for TNF-α

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	5	285	-0.06 pg/mL [-0.12, 0.01]	Chi² = 0.21, df = 1 (P = 0.65), I² = 0%
	More than three months	3	197	-0.70 pg/mL [-3.48, 2.08]	
Risk of bias	Low/unclear	2	148	0.11 pg/mL [-0.51, 0.73]	Chi² = 0.21, df = 1 (P = 0.65), I² = 0%
	High	6	334	-0.04 pg/mL [-0.22, 0.15]	
Nut type	Almond	3	151	-0.06 pg/mL [-0.13, 0.01]	Chi² = 6.75, df = 4 (P = 0.15), I² = 40.8%
	Walnut	2	90	-0.03 pg/mL [-0.21, 0.14]	
	Mixed nut	1	108	0.70 pg/mL [-0.41, 1.81]	
	Peanut	1	65	-0.16 pg/mL [-1.41, 1.10]	
	Pistachio	1	68	-3.70 pg/mL [-6.93, -0.47]	
Health status	Healthy	1	40	-0.01 pg/mL [-0.24, 0.22]	Chi² = 7.08, df = 5 (P = 0.21), I² =

	Chronic disease risk factors	2	115	-0.07 pg/mL [-0.34, 0.20]	29.4%
	T2DM	2	61	-0.06 pg/mL [-0.13, 0.01]	
	MetS	1	68	-3.70 pg/mL [-6.93, -0.47]	
	CAD	1	90	0.10 pg/mL [-0.54, 0.74]	
	Combination	1	108	0.70 pg/mL [-0.41, 1.81]	
Energy value of nuts included in diet	Adjusted	6	421	-0.04 pg/mL [-0.24, 0.15]	Chi^2 = 0.05, df = 1 (P = 0.83), I^2 = 0%
	Not adjusted	2	61	-0.01 pg/mL [-0.24, 0.22]	
Study design	Parallel	4	262	-0.27 pg/mL [-1.68, 1.14]	Chi^2 = 0.09, df = 1 (P = 0.77), I^2 = 0%
	Cross-over	4	220	-0.05 pg/mL [-0.12, 0.01]	
Nut dose	<50g/day	5	287	-0.02 pg/mL [-0.34, 0.31]	Chi^2 = 0.06, df = 1 (P = 0.80), I^2 = 0%
	≥50g/day	3	195	-0.06 pg/mL [-0.13, 0.01]	

Table 5: Results of sub-group analyses for IL-6

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	7	386	0.04 pg/mL [-0.02, 0.09]	Chi² = 2.71, df = 1 (P = 0.10), I² = 63.1%
	More than three months	6	520	-0.19 pg/mL [-0.45, 0.07]	
Risk of bias	Low/unclear	5	314	-0.01 pg/mL [-0.26, 0.23]	Chi² = 0.62, df = 1 (P = 0.43), I² = 0%
	High	8	592	-0.13 pg/mL [-0.29, 0.03]	
Nut type	Almond	4	201	-0.16 pg/mL [-0.44, 0.13]	Chi² = 5.17, df = 4 (P = 0.27), I² = 22.6%
	Walnut	3	216	-0.11 pg/mL [-0.31, 0.10]	
	Hazelnut	2	163	0.05 pg/mL [-0.01, 0.11]	
	Mixed nut	3	218	-0.18 pg/mL [-0.99, 0.63]	
	Pistachio	1	108	-0.14 pg/mL [-0.47, 0.19]	
Health status	Chronic disease risk	6	497	0.04 pg/mL [-0.02, 0.10]	Chi² = 3.09, df = 5 (P = 0.69), I² = 0%

	factors				
	Healthy	1	40	-0.10 pg/mL [-0.39, 0.19]	
	MetS	2	110	-0.47 pg/mL [-2.44, 1.49]	
	T2DM	2	61	-0.14 pg/mL [-0.46, 0.18]	
	CAD	1	90	-0.50 pg/mL [-1.62, 0.62]	
	Combination	1	108	0.00 pg/mL [-0.41, 0.41]	
Energy value of nuts included in diet	Adjusted	8	628	0.03 pg/mL [-0.02, 0.09]	Chi^2 = 0.68, df = 1 (P = 0.41), I^2 = 0%
	Not adjusted	5	278	-0.18 pg/mL [-0.68, 0.32]	
Study design	Parallel	7	528	-0.04 pg/mL [-0.29, 0.22]	Chi^2 = 0.26, df = 1 (P = 0.61), I^2 = 0%
	Cross-over	6	378	-0.12 pg/mL [-0.27, 0.04]	
Nut dose	<50g/day	9	618	-0.03 pg/mL [-0.17, 0.12]	Chi^2 = 0.65, df = 1 (P = 0.42), I^2 = 0%
	≥50g/day	4	288	-0.14 pg/mL [-0.36, 0.09]	

Table 6: Results of sub-group analyses for ICAM-1

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	12	537	0.66 ng/mL [-0.56, 1.88]	$Chi^2 = 0.04$, df = 1 (P = 0.83), I^2 = 0%
	More than three months	3	510	2.35 ng/mL [-13.26, 17.96]	
Risk of bias	Low/unclear	8	660	4.58 ng/mL [-2.68, 11.85]	$Chi^2 = 1.14$, df = 1 (P = 0.29), I^2 = 12.4%
	High	7	387	0.57 ng/mL [-0.66, 1.80]	
Nut type	Almond	3	81	11.65 ng/mL [-1.49, 24.80]	$Chi^2 = 3.34$, df = 4 (P = 0.50), I^2 = 0%
	Walnut	5	244	0.58 ng/mL [-0.65, 1.81]	
	Hazelnut	2	163	-3.32 ng/mL [-22.42, 15.78]	
	Mixed nut	4	499	3.75 ng/mL [-7.31, 14.81]	
	Pistachio	1	60	-2.60 ng/mL [-18.13, 12.93]	

Health status	Healthy	1	40	0.65 ng/mL [-0.59, 1.89]	Chi² = 1.02, df = 4 (P = 0.91), I² = 0%
	Chronic disease risk factors	9	444	0.86 ng/mL [-6.94, 8.65]	
	T2DM	2	100	-1.67 ng/mL [-16.50, 13.16]	
	MetS	2	110	-13.46 ng/mL [-76.61, 49.70]	
	Combination	1	353	8.00 ng/mL [-8.85, 24.85]	
Energy value of nuts included in diet	Adjusted	9	749	-1.31 ng/mL [-8.90, 6.29]	Chi² = 0.48, df = 1 (P = 0.49), I² = 0%
	Not adjusted	6	298	2.06 ng/mL [-3.72, 7.84]	
Study design	Parallel	7	667	5.39 ng/mL [-2.46, 13.24]	Test for subgroup differences: Chi² = 1.42, df = 1 (P = 0.23), I² = 29.6%
	Cross-over	8	380	0.56 ng/mL [-0.66, 1.79]	
Nut dose	<50g/day	9	830	0.62 ng/mL [-0.60, 1.84]	Chi² = 0.29, df = 1 (P = 0.59), I² = 0%
	≥50g/day	6	217	3.66 ng/mL [-7.32, 14.65]	

Table 7: Results of sub-group analyses for VCAM-1

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	11	537	2.23 ng/mL [-9.68, 14.13]	$Chi^2 = 0.02$, df = 1 (P = 0.89), I^2 = 0%
	More than three months	3	267	-4.16 ng/mL [-96.76, 88.44]	
Risk of bias	Low/unclear	8	417	2.39 ng/mL [-9.72, 14.50]	$Chi^2 = 0.04$, df = 1 (P = 0.83), I^2 = 0%
	High	6	387	7.42 ng/mL [-38.20, 53.04]	
Nut type	Almond	4	171	1.11 ng/mL [-13.10, 15.33]	$Chi^2 = 1.56$, df = 4 (P = 0.82), I^2 = 0%
	Walnut	3	154	-30.19 ng/mL [-99.92, 39.53]	
	Hazelnut	2	163	17.62 ng/mL [-24.61, 59.85]	
	Mixed nut	4	256	9.30 ng/mL [-21.20, 39.80]	
	Pistachio	1	60	3.40 ng/mL [-60.84, 67.64]	

Health status	Chronic disease risk factors	8	394	3.95 ng/mL [-9.12, 17.02]	Chi² = 2.08, df = 4 (P = 0.72), I² = 0%
	T2DM	2	100	-17.58 ng/mL [-67.98, 32.82]	
	MetS	2	110	9.61 ng/mL [-23.37, 42.59]	
	CAD	1	90	-48.00 ng/mL [-193.52, 97.52]	
	Combination	1	110	-70.00 ng/mL [-230.43, 90.43]	
Energy value of nuts included in diet	Adjusted	9	546	-12.78 ng/mL [-42.38, 16.83]	Chi² = 1.27, df = 1 (P = 0.26), I² = 21.0%
	Not adjusted	5	258	5.71 ng/mL [-7.00, 18.42]	
Study design	Parallel	7	424	5.01 ng/mL [-7.27, 17.29]	Chi² = 1.26, df = 1 (P = 0.26), I² = 20.5%
	Cross-over	7	380	-17.66 ng/mL [-55.33, 20.02]	
Nut dose	<50g/day	7	497	9.74 ng/mL [-14.01, 33.49]	Chi² = 0.43, df = 1 (P = 0.51), I² = 0%
	≥50g/day	7	307	0.63 ng/mL [-12.78, 14.04]	

Supplementary material 5: Forest plots of difference in CRP after exclusion of individual studies

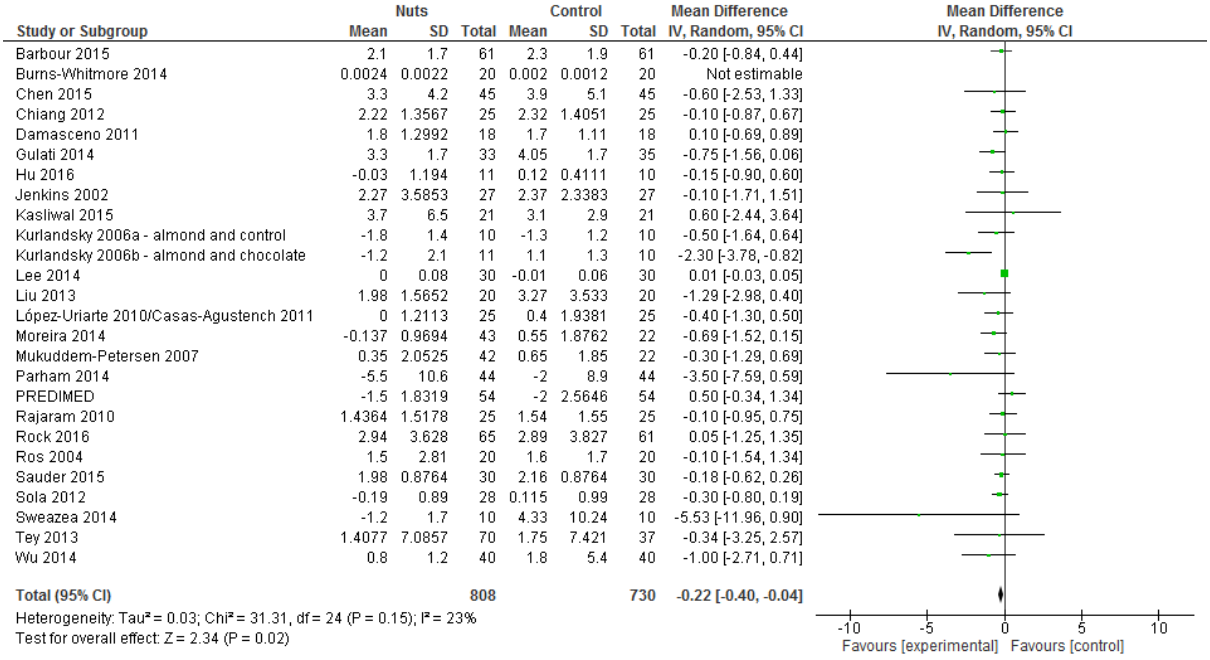

Figure 1: Difference in CRP (mg/L) between nut consumption and control, after exclusion of Burns-Whitmore et al. (2014). Diamond indicates weighted mean difference with 95% confidence intervals.

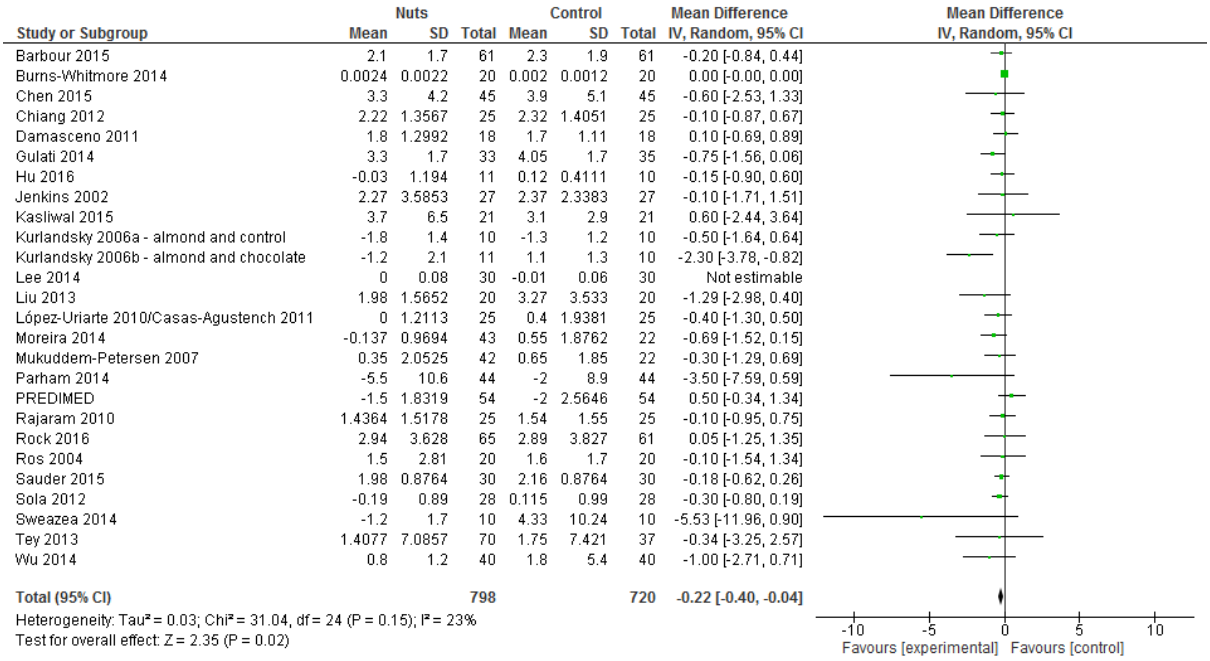

Figure 2: Difference in CRP (mg/L) between nut consumption and control, after exclusion of Lee et al. (2014). Diamond indicates weighted mean difference with 95% confidence intervals.

Supplementary material 6: Forest plots of differences in biomarkers between nut consumption and control

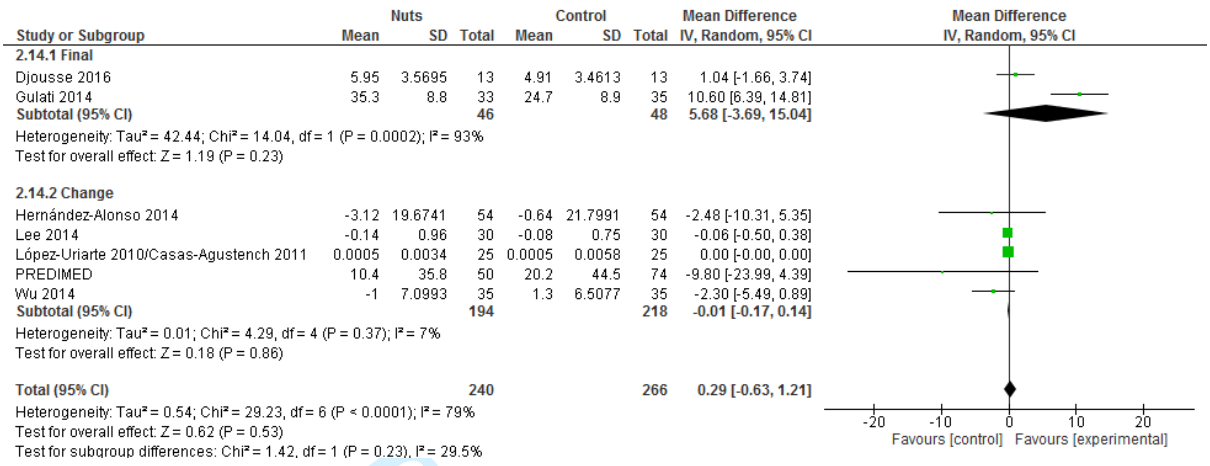

Figure 3: Difference in adiponectin (µg/mL) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals.

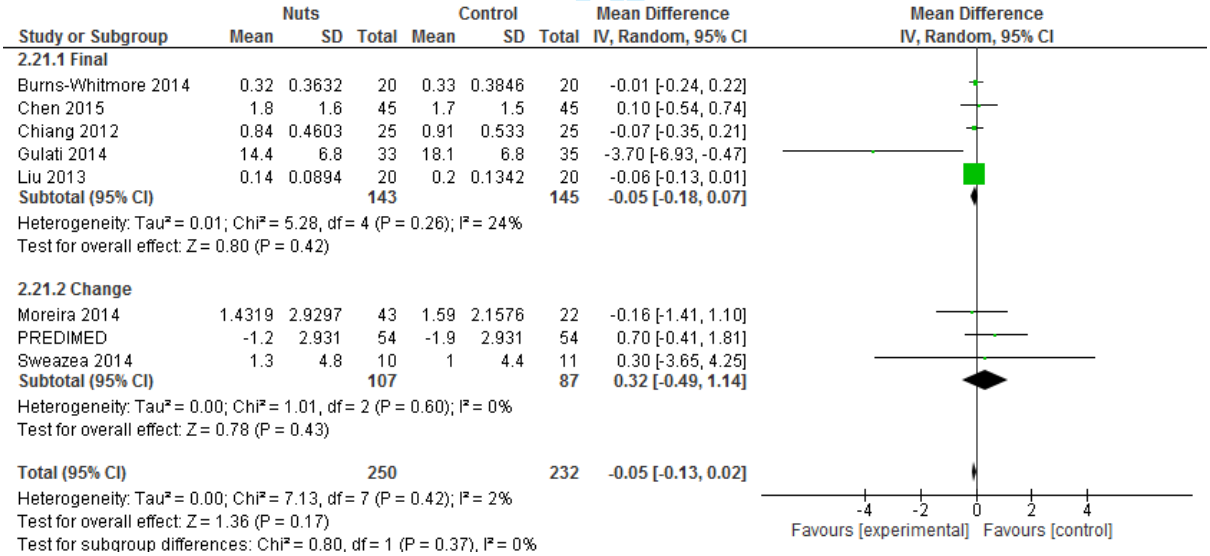

Figure 4: Difference in TNF-α (pg/mL) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals.

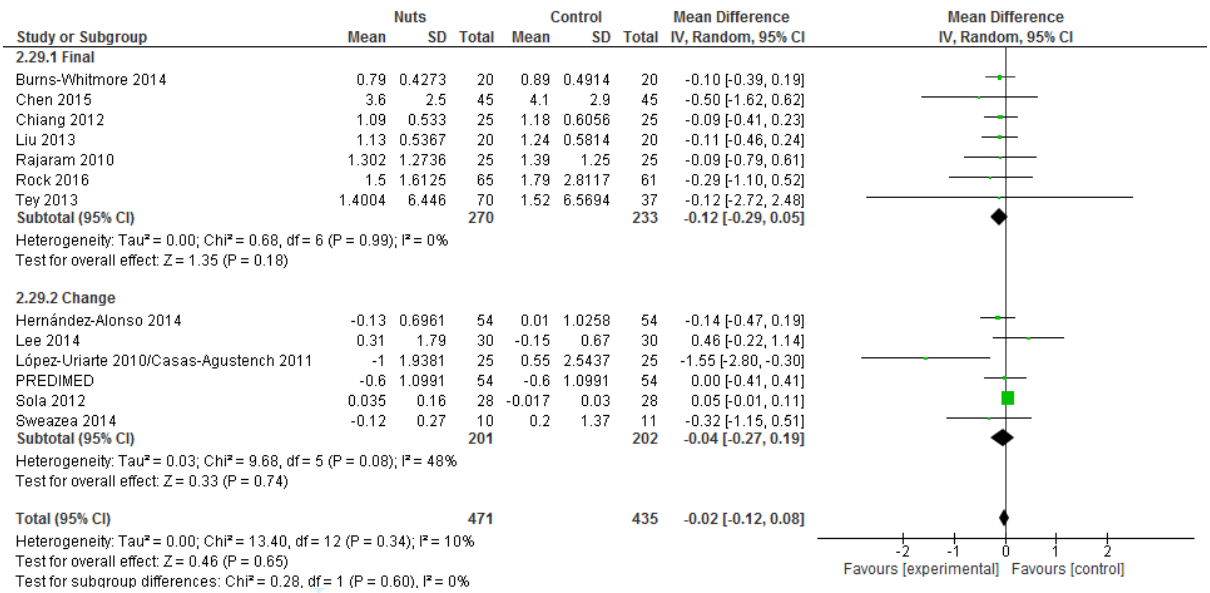

Figure 5: Difference in IL-6 (pg/mL) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals

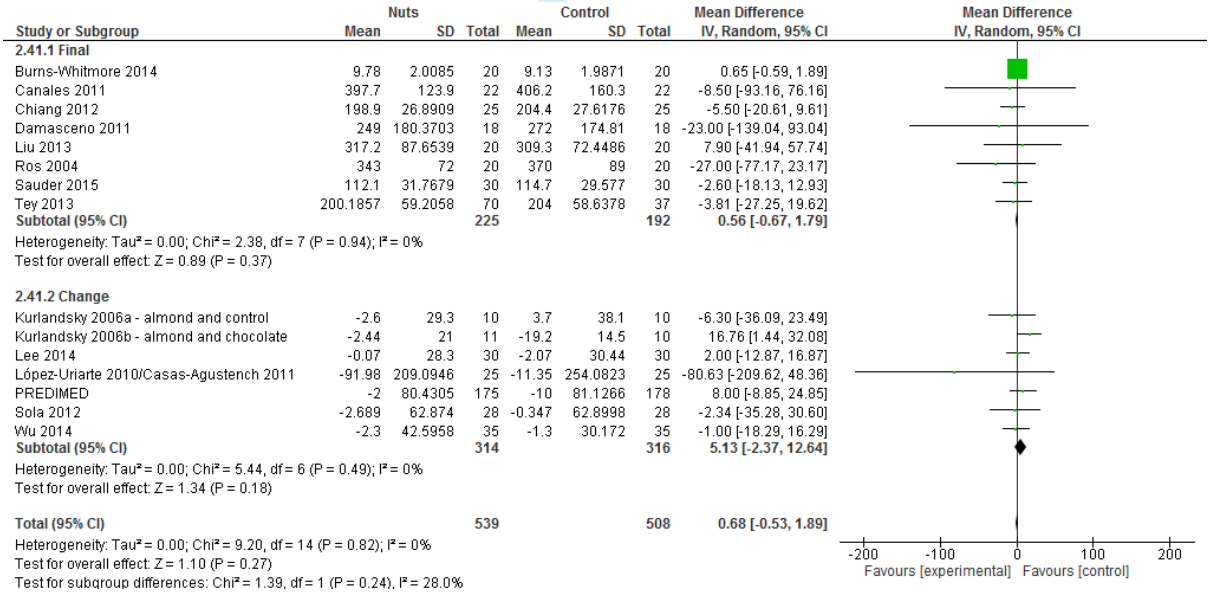

Figure 6: Difference in ICAM-1 (ng/mL) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals

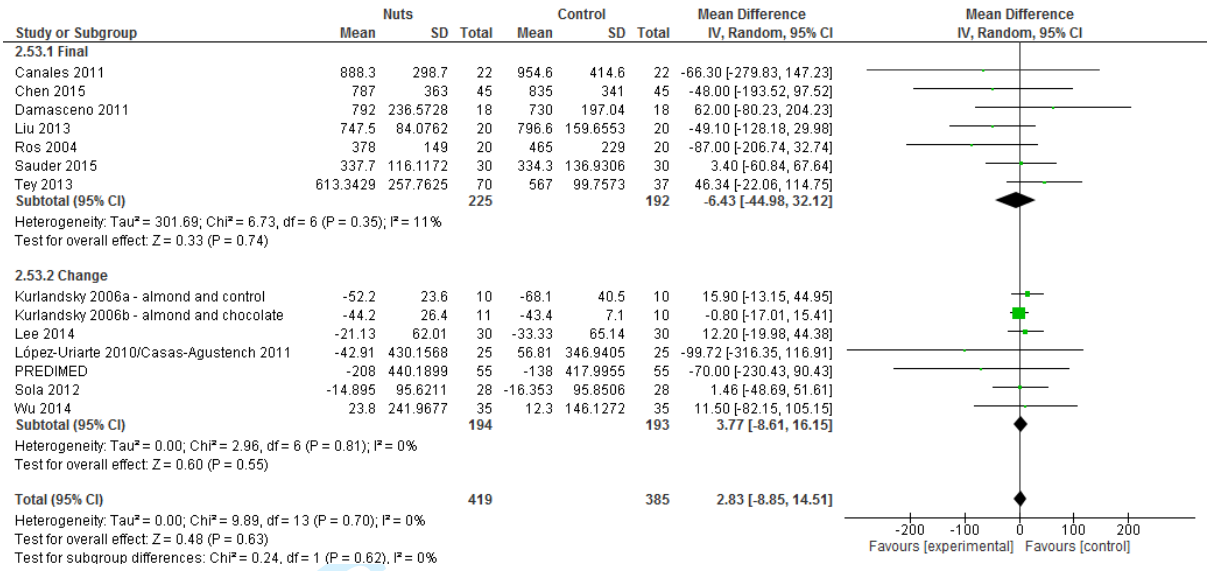

Figure 7: Difference in VCAM-1 (ng/mL) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals

Supplementary material 7: Funnel plots

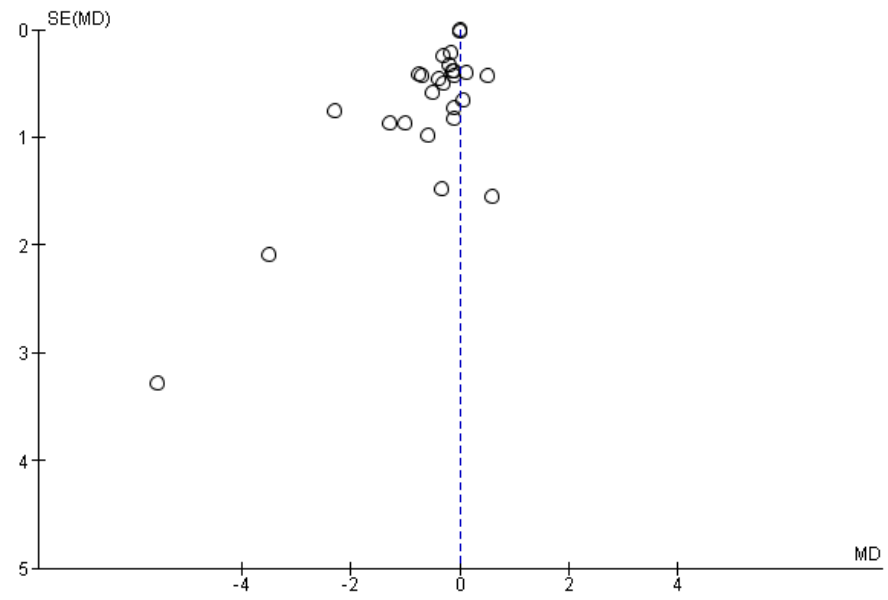

Figure 8: Funnel plot of the effect of nut consumption on CRP (mg/L)

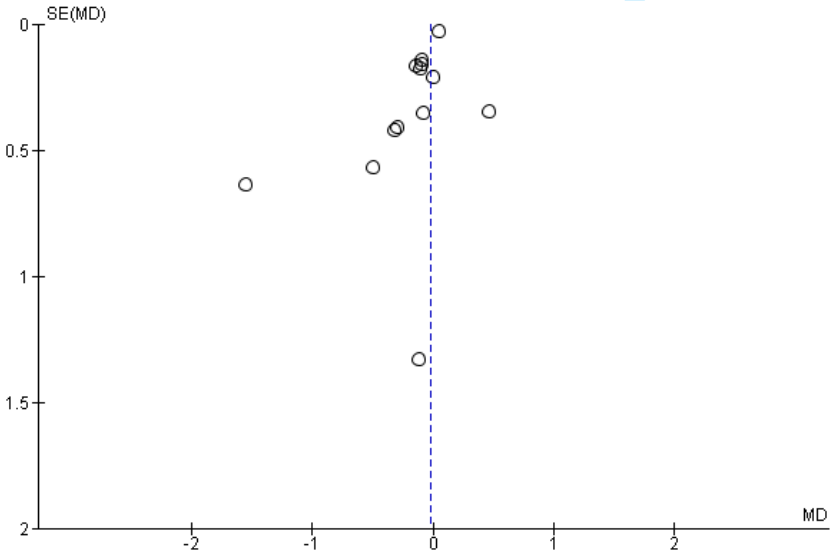

Figure 9: Funnel plot of the effect of nut consumption on IL-6 (pg/mL)

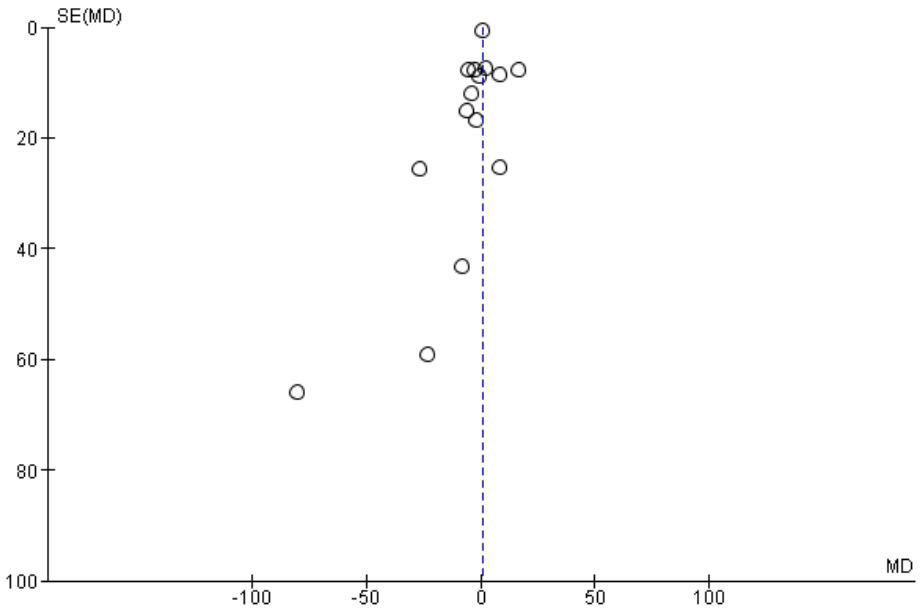

Figure 10: Funnel plot of the effect of nut consumption on ICAM-1 (ng/mL)

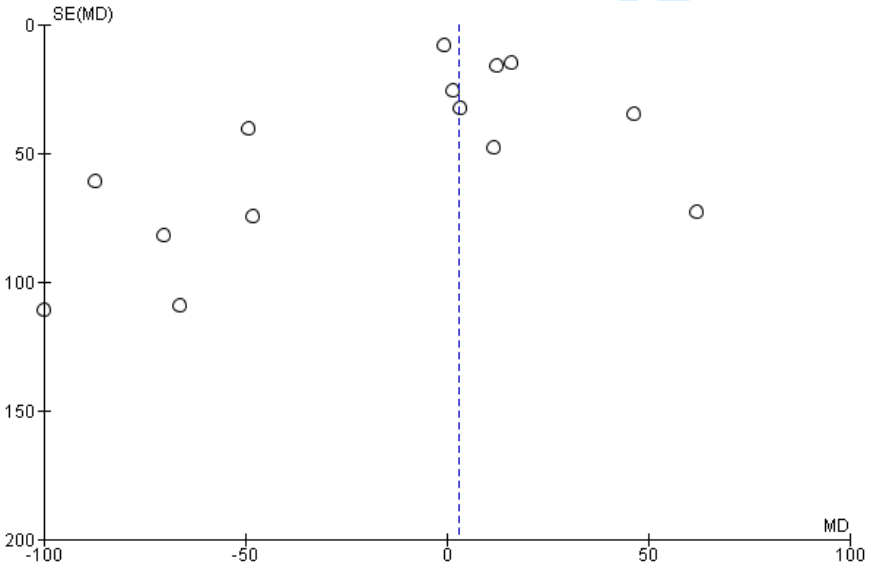

Figure 11: Funnel plot of the effect of nut consumption on VCAM-1 (ng/mL)

Supplementary material 8: Risk of bias assessment summary

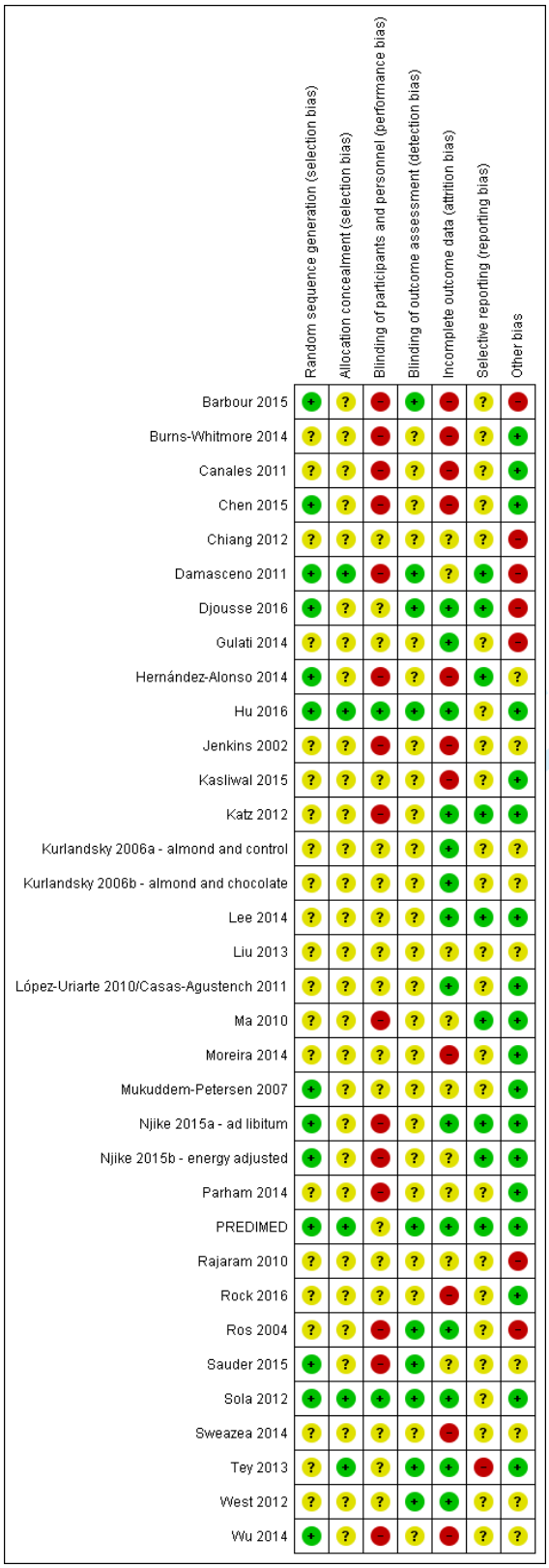

Figure 12: Risk of bias assessment for each study

Supplementary material 9: Justification for risk of bias judgements

Barbour et al., 2015

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Article states: "Subjects were randomised using computer generated software"
Allocation concealment (selection bias)	Unclear risk	Not specified
Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Low risk	Article states: "Data entry and analysis was blinded to minimise investigator bias"
Incomplete outcome data (attrition bias)	High risk	>10% withdrawal, intention-to-treat (ITT) not used
Selective reporting (reporting bias)	Unclear risk	ANZCTRN registration available, includes pre-specified outcomes not reported in this paper but which may have been reported in unpublished primary paper
Other bias	High risk	No washout period - authors specify 12 week period would have been sufficient to avoid carry over effects but this is not clear

Burns-Whitmore et al., 2014

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, method not given
Allocation concealment (selection bias)	Unclear risk	Not specified

Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although outcomes unlikely to be influenced by blinding
Incomplete outcome data (attrition bias)	High risk	>20% withdrawal, ITT not used (not clear which group participants dropped out of)
Selective reporting (reporting bias)	Unclear risk	Protocol not available
Other bias	Low risk	4 week wash-out period (justified). Did not report baseline results for outcomes of interest, but unlikely to influence as cross-over study

Canales et al., 2011

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, method not given
Allocation concealment (selection bias)	Unclear risk	Not specified
Blinding of participants and personnel (performance bias)	High risk	Stated to be non-blinded. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although outcomes unlikely to be influenced by blinding
Incomplete outcome data (attrition bias)	High risk	>10% withdrawal, ITT not used
Selective reporting (reporting bias)	Unclear risk	Protocol not available
Other bias	Low risk	4 -6 week wash-out period (appears suitable)

Chen et al., 2015

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	The program in the randomization.com was employed for the randomization
Allocation concealment (selection bias)	Unclear risk	Not specified
Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although outcomes unlikely to be influenced by blinding
Incomplete outcome data (attrition bias)	High risk	>10% withdrawal, ITT not used
Selective reporting (reporting bias)	Unclear risk	Clinical trial registration provides insufficient detail to determine if all outcomes reported
Other bias	Low risk	Wash-out period of 4 weeks appears suitable

Chiang et al., 2012

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, method not given
Allocation concealment (selection bias)	Unclear risk	Not specified
Blinding of participants and personnel (performance bias)	Unclear risk	single-blinded, unclear who was blinded (participants vs personnel) as all foods provided
Blinding of outcome assessment (detection bias)	Unclear risk	Stated to be single-blind (assume outcome assessors), outcomes unlikely to be influenced by blinding
Incomplete outcome data (attrition bias)	Unclear risk	<10%, however unclear at which point withdrew
Selective reporting (reporting bias)	Unclear risk	Protocol not available

Other bias	High risk	Wash-out period of 2 days

Damasceno et al., 2011

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Randomization was simple (not stratified) and was based on a random number table prepared by a biostatistician
Allocation concealment (selection bias)	Low risk	"...six possible diet sequences, which were coded and introduced into sealed envelopes"
Blinding of participants and personnel (performance bias)	High risk	Stated as not possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Low risk	Investigators involved in preparation of databases and laboratory determinations, however, were masked with respect to treatment sequence
Incomplete outcome data (attrition bias)	Unclear risk	<10%, however unclear at which point withdrew
Selective reporting (reporting bias)	Low risk	The study protocol is available and all pre-specified outcomes of interest to the review have been reported in the pre-specified way
Other bias	High risk	No washout period. Authors state would not effect, but likely to be carry-over effect

Djousse et al., 2016

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Article states: "computer-generated randomization schedule with balanced blocks, stratified by prevalent DM and coronary artery disease"

Allocation concealment (selection bias)	Unclear risk	Biostatistician generated schedule and did not have contact with study subjects, but not clear how allocation was communicated to researchers
Blinding of participants and personnel (performance bias)	Unclear risk	Unclear if participants blinded, researcher providing intervention not blinded
Blinding of outcome assessment (detection bias)	Low risk	Test completed by blinded staff
Incomplete outcome data (attrition bias)	Low risk	<5% withdrawal
Selective reporting (reporting bias)	Low risk	The study protocol is available and all pre-specified outcomes of interest to the review have been reported in the pre-specified way
Other bias	High risk	Control group had significantly higher proportion with hypercholesterolaemia

Gulati et al., 2014

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, however no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	No details given
Blinding of participants and personnel (performance bias)	Unclear risk	Not stated if participants blinded, would not be possible to blind personnel
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	Low risk	12% drop-out, but similar between groups and ITT used
Selective reporting (reporting bias)	Unclear risk	protocol not available
Other bias	High risk	CRP significantly higher in control group at baseline

Hernández-Alonso et al., 2014

Bias	**Authors' judgement**	**Support for judgement**

Random sequence generation (selection bias)	Low risk	Article states: "randomly assigned to one of the two different intervention periods using a computer generated random number table"
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, however would be unlikely to affect results
Incomplete outcome data (attrition bias)	High risk	10% drop-out (ITT used) - but all dropped out during first pistachio sequence
Selective reporting (reporting bias)	Low risk	The study protocol is available and all pre-specified outcomes of interest to the review have been reported in the pre-specified way
Other bias	Unclear risk	2 week washout period, unclear if sufficient

Hu et al., 2016

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Randomisation sequence was computer generated
Allocation concealment (selection bias)	Low risk	Study states: "Allocation concealment was achieved by keeping codes in a sealed envelope by a person who was not in contact with study subjects, and codes were disclosed after the study"
Blinding of participants and personnel (performance bias)	Low risk	Study states: "It was impossible to blind participants because of the nature of the intervention (especially the Brazil nuts), but all data curation, checking, measurements and data analysis were conducted by researchers blinded to treatment allocation of subjects."

Blinding of outcome assessment (detection bias)	Low risk	Study states: "It was impossible to blind participants because of the nature of the intervention (especially the Brazil nuts), but all data curation, checking, measurements and data analysis were conducted by researchers blinded to treatment allocation of subjects."
Incomplete outcome data (attrition bias)	Low risk	<10% drop-out and evenly spread between groups
Selective reporting (reporting bias)	Unclear risk	Protocol available, but not possible to determine if all outcomes reported
Other bias	Low risk	

Jenkins et al., 2002

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, however would be unlikely to affect results
Incomplete outcome data (attrition bias)	High risk	>20% drop-out, and unclear at which point in study participants dropped out
Selective reporting (reporting bias)	High risk	Study protocol is available but unclear if all relevant outcomes have not been reported
Other bias	Unclear risk	2 week washout period, unclear if sufficient

Kasliwal et al., 2015

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	Unclear risk	"open-label", unclear if both participants and personnel unblinded
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	High risk	>20% drop-out rate, ITT not used
Selective reporting (reporting bias)	Unclear risk	protocol not available
Other bias	Low risk	

Katz et al., 2012

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Unclear risk	Single-blinded (unclear who was blinded though), although would be unlikely to affect results
Incomplete outcome data (attrition bias)	Low risk	13% dropout (ITT used), but similar between groups
Selective reporting (reporting bias)	Low risk	The study protocol is available and all pre-specified outcomes of interest to the review have been reported in the pre-specified way

Other bias	Low risk	Wash-out period of 4 weeks appears suitable

Kurlandsky 2006a - almond and control

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	Unclear risk	Not possible to blind personnel, unclear if participants blinded
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	Low risk	<5% dropout, although not clear which group dropped out of
Selective reporting (reporting bias)	Unclear risk	protocol not available
Other bias	Unclear risk	Age differed significantly between groups, unclear if impacted on results

Kurlandsky 2006b - almond and chocolate

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	Unclear risk	Not possible to blind personnel, unclear if participants blinded
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	Low risk	<5% dropout, although not clear which group dropped out of
Selective reporting (reporting bias)	Unclear risk	protocol not available

Other bias	Unclear risk	Age differed significantly between groups, unclear if impacted on results

Lee et al., 2014

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	Unclear risk	Not possible to blind personnel, unclear if participants blinded
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	Low risk	<5% dropout, group specified
Selective reporting (reporting bias)	Low risk	The study protocol is available and all pre-specified outcomes of interest to the review have been reported in the pre-specified way
Other bias	Low risk	No differences in baseline characteristics

Liu et al., 2013

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	Unclear risk	Unclear if blinded as all foods provided
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	Unclear risk	<10% dropout, but unclear during which diet participant dropped out

Selective reporting (reporting bias)	Unclear risk	protocol not available
Other bias	Unclear risk	2 week washout period, unclear if sufficient

López-Uriarte et al., 2010/Casas-Agustench et al., 2011

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, method not given
Allocation concealment (selection bias)	Unclear risk	Not specified
Blinding of participants and personnel (performance bias)	Unclear risk	Not possible to blind personnel, unclear if participants blinded
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although outcomes unlikely to be influenced by blinding
Incomplete outcome data (attrition bias)	Low risk	<5% withdrawal
Selective reporting (reporting bias)	Unclear risk	Clinical trial registration provides insufficient detail to determine if all outcomes reported
Other bias	Low risk	

Ma et al., 2010

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods

Blinding of outcome assessment (detection bias)	Unclear risk	Single-blinded (unclear if all outcome assessors blinded), although would be unlikely to affect results
Incomplete outcome data (attrition bias)	Unclear risk	<10% dropout, ITT used (although unclear when participants dropped out)
Selective reporting (reporting bias)	Low risk	The study protocol is available and all pre-specified outcomes of interest to the review have been reported in the pre-specified way
Other bias	Low risk	8 week washout appears adequate

Moreira et al., 2014

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	Unclear risk	Not possible to blind personnel, unclear if participants blinded
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	High risk	>10% drop out/excluded, not evenly spread across groups
Selective reporting (reporting bias)	Unclear risk	protocol not available
Other bias	Low risk	

Mukuddem-Petersen et al., 2007

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Drawing numbers from a hat
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	Unclear risk	Not possible to blind personnel, unclear if participants blinded

Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	Unclear risk	<10% drop-out, but unclear during which diet participants dropped out
Selective reporting (reporting bias)	Unclear risk	protocol not available
Other bias	Low risk	

Njike et al., 2015a – non-calorie adjusted

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	study participants were randomized using a SAS-generated random table
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, however would be unlikely to affect results
Incomplete outcome data (attrition bias)	Low risk	>10% drop-out, but ITT and similar between groups
Selective reporting (reporting bias)	Low risk	The study protocol is available and all pre-specified outcomes of interest to the review have been reported in the pre-specified way
Other bias	Low risk	

Njike et al., 2015b – calorie adjusted

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	study participants were randomized using a SAS-generated random table

Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, however would be unlikely to affect results
Incomplete outcome data (attrition bias)	Unclear risk	14% drop-out (ITT used) but 3 x in walnut arm
Selective reporting (reporting bias)	Low risk	The study protocol is available and all pre-specified outcomes of interest to the review have been reported in the pre-specified way
Other bias	Low risk	

Parham et al., 2014

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Allocation based on random numbers, but not clear how generated
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	Unclear risk	<10%, but not clear when participants withdrew/were excluded
Selective reporting (reporting bias)	Unclear risk	protocol not available

Other bias	Low risk	washout period of 8 weeks appears appropriate

PREDIMED

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Article states: "Randomization was performed centrally by means of a computer-generated random-number sequence"
Allocation concealment (selection bias)	Low risk	"These tables have been centrally elaborated by the Coordinating Unit and provide a stratified random sequence of allocation for each FC using closed envelopes"
Blinding of participants and personnel (performance bias)	Unclear risk	single-blinded
Blinding of outcome assessment (detection bias)	Low risk	Outcome assessors blinded
Incomplete outcome data (attrition bias)	Low risk	participants completers only
Selective reporting (reporting bias)	Low risk	The study protocol is available and all pre-specified outcomes of interest to the review have been reported in the pre-specified way
Other bias	Low risk	

Rajaram et al., 2010

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	3 x 3 Latin square design, no description of method of randomisation
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	Unclear risk	single-blinded, unclear if participants aware as all foods provided

Blinding of outcome assessment (detection bias)	Unclear risk	single-blind (not stated who blinded), although would be unlikely to affect results
Incomplete outcome data (attrition bias)	Unclear risk	<10%, but not clear when participants withdrew/were excluded
Selective reporting (reporting bias)	Unclear risk	protocol not available
Other bias	High risk	washout period not included, Sabate paper states lipids would stabilise but would still impact starting levels

Rock et al., 2016

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Randomised by study statistician, not clear if involved in other aspects of study
Blinding of participants and personnel (performance bias)	Unclear risk	Not possible to blind personnel, unclear if participants blinded
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	High risk	18% withdrawal, does not appear that ITT used for biomarkers analysis (Table 3)
Selective reporting (reporting bias)	Unclear risk	Protocol is available, but insufficient detail to determine if all outcomes reported
Other bias	Low risk	

Ros et al., 2004

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Randomised but no additional detail given
Allocation concealment (selection bias)	Unclear risk	Not stated

Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention
Blinding of outcome assessment (detection bias)	Low risk	Blinded
Incomplete outcome data (attrition bias)	Low risk	<5% dropout (although not clear when dropped out)
Selective reporting (reporting bias)	Unclear risk	protocol not available
Other bias	High risk	washout period not included, references paper stating lipids would stabilise but would still

Sauder et al., 2015

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Generated via randomization.com
Allocation concealment (selection bias)	Unclear risk	Generated by study coordinator, but not stated if concealed
Blinding of participants and personnel (performance bias)	High risk	"But due to the nature of the dietary intervention, participants were aware of their treatment order assignment"
Blinding of outcome assessment (detection bias)	Low risk	Technicians who measured outcome variables were blinded to treatment assignments
Incomplete outcome data (attrition bias)	Unclear risk	11.7% drop-out, but not clear when participants dropped out
Selective reporting (reporting bias)	Unclear risk	Protocol is available, but insufficient detail to determine if all outcomes reported
Other bias	Unclear risk	washout period of 2 weeks

Sola et al., 2012

Bias	**Authors' judgement**	**Support for judgement**

Random sequence generation (selection bias)	Low risk	The randomization code was computer-generated random number sequence in gender-stratified blocks
Allocation concealment (selection bias)	Low risk	Center and treatment assignment codes were allocated via an interactive electronic response system administered by the Barcelona Randomization Unit, which was not further involved in the study.
Blinding of participants and personnel (performance bias)	Low risk	The participants, clinical investigators and laboratory personnel were blinded with respect to the type of cream being consumed
Blinding of outcome assessment (detection bias)	Low risk	The participants, clinical investigators and laboratory personnel were blinded with respect to the type of cream being consumed
Incomplete outcome data (attrition bias)	Low risk	<10% dropout, similar between groups, ITT used
Selective reporting (reporting bias)	Unclear risk	Protocol is available, but insufficient detail to determine if all outcomes reported
Other bias	Low risk	No differences in baseline characteristics

Sweazea et al., 2014

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	Unclear risk	Not possible to blind personnel, unclear if participants blinded
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	High risk	>10% drop out, ITT not used
Selective reporting (reporting bias)	Unclear risk	protocol not available
Other bias	Unclear risk	Unclear if baseline inflammation levels differ between groups

Tey et al., 2013

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Details of randomisation given, but not how sequence was generated
Allocation concealment (selection bias)	Low risk	Managed by an off-site statistician
Blinding of participants and personnel (performance bias)	Unclear risk	Not possible to blind personnel, unclear if participants blinded
Blinding of outcome assessment (detection bias)	Low risk	Stated to be blinded
Incomplete outcome data (attrition bias)	Low risk	5% drop-out, ITT used, similar drop-out between groups
Selective reporting (reporting bias)	High risk	TNF-α referenced in protocol, not reported in paper.
Other bias	Low risk	controlled for baseline values

West et al., 2012

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, but no further detail given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	Unclear risk	Unclear if blinded as all foods provided
Blinding of outcome assessment (detection bias)	Low risk	Appears to be blinded (Gebauer et al., 2008)
Incomplete outcome data (attrition bias)	Low risk	<5% drop-out (although not clear which group dropped out of)
Selective reporting (reporting bias)	Unclear risk	Protocol not available
Other bias	Unclear risk	2 weeks compliance break (assume washout)

Wu et al., 2014

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	computer generated randomisation sequence

Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	High risk	~20% drop-out
Selective reporting (reporting bias)	Unclear risk	Protocol available, but not possible to determine if all outcomes reported
Other bias	Unclear risk	2 weeks washout

Supplementary material 10: GRADE assessment of the quality of the body of evidence

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	nut consumption	control	Relative (95% CI)	Absolute (95% CI)		
CRP												
26	randomised trials	serious [a]	not serious [b]	not serious	not serious	publication bias strongly suspected [c]	828	750	-	MD **0.01 lower** (0.06 lower to 0.03 higher)	⊕⊕◯◯ LOW	IMPORTANT
Adiponectin												
7	randomised trials	serious [d]	serious [e]	not serious	serious [f]	none	240	266	-	MD **0.29 higher** (0.63 lower to 1.21 higher)	⊕◯◯◯ VERY LOW	IMPORTANT
TNF-a												
8	randomised trials	serious [g]	not serious	not serious	not serious	none	250	232	-	MD **0.05 lower** (0.13 lower to 0.02 higher)	⊕⊕⊕◯ MODERATE	IMPORTANT
IL-6												
13	randomised trials	serious [h]	not serious	not serious	not serious	publication bias strongly suspected [i]	471	435	-	MD **0.02 lower** (0.12 lower to 0.08 higher)	⊕⊕◯◯ LOW	IMPORTANT
ICAM-1												

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	nut consumption	control	Relative (95% CI)	Absolute (95% CI)		
15	randomised trials	not serious [i]	not serious	not serious	not serious	none	539	508	-	MD **0.68 higher** (0.53 lower to 1.89 higher)	⨁⨁⨁⨁ HIGH	IMPORTANT
VCAM-1												
14	randomised trials	not serious [k]	not serious	not serious	not serious	none	419	385	-	MD **2.83 higher** (8.85 lower to 14.51 higher)	⨁⨁⨁⨁ HIGH	IMPORTANT
FMD												
9	randomised trials	not serious [l]	not serious	not serious	not serious	none	326	326	-	MD **0.79 higher** (0.35 higher to 1.23 higher)	⨁⨁⨁⨁ HIGH	IMPORTANT

CI: Confidence interval; **MD:** Mean difference

a. The studies were viewed as being in the category of 'serious limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'high risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'high risk' needed to be categorised as either 'serious limitations' or 'very serious limitations'. In view of the potential implications of the 'high risk' aspects on the quality of the body of evidence, 'serious limitations' was selected

b. I squared value of 20%, indicating minimal heterogeneity

c. Funnel plot indicates likelihood of publication bias

d. The studies were viewed as being in the category of 'serious limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'high risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'high risk' needed to be categorised as either 'serious limitations' or 'very serious limitations'. In view of the potential implications of the 'high risk' aspects on the quality of the body of evidence, 'serious limitations' was selected

e. I squared value of 79% indicating considerable heterogeneity

f. Total sample size is greater than 400, however 95% CIs overlap no effect and include appreciable benefit or harm

g. The studies were viewed as being in the category of 'serious limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'high risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'high risk' needed to be categorised as either 'serious limitations' or 'very serious limitations'. In view of the potential implications of the 'high risk' aspects on the quality of the body of evidence, 'serious limitations' was selected

h. The studies were viewed as being in the category of 'serious limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'high risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'high risk' needed to be categorised as either 'serious limitations' or 'very serious limitations'. In view of the potential implications of the 'high risk' aspects on the quality of the body of evidence, 'serious limitations' was selected

i. Funnel plot indicates likelihood of publication bias

j. The studies were viewed as being in the category of 'no limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'unclear risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'unclear risk' needed to be categorised as either 'no limitations' or 'serious limitations'. In view of the potential implications of the 'unclear risk' aspects on the quality of the body of evidence, 'no limitations' was selected

k. The studies were viewed as being in the category of 'no limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'unclear risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'unclear risk' needed to be categorised as either 'no limitations' or 'serious limitations'. In view of the potential implications of the 'unclear risk' aspects on the quality of the body of evidence, 'no limitations' was selected

l. The studies were viewed as being in the category of 'no limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'unclear risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'unclear risk' needed to be categorised as either 'no limitations' or 'serious limitations'. In view of the potential implications of the 'unclear risk' aspects on the quality of the body of evidence, 'no limitations' was selected

Supplementary material 1: PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	2
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5 -6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5 -6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supplementary material 2
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	7

Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	8,9
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7-8

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	8,9
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Supplementary material 8, 9
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Table 2, Figure 2, Figure 3, Supplementary material 6
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Table 2
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Figure 4
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Table 2, Supplementary material 3, 4, 5

DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	24 - 30
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	28 - 30
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	30
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	31

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

Page 2 of 2

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The effect of nut consumption on markers of inflammation and endothelial function: a systematic review and meta-analysis of randomised controlled trials

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Title: The effect of nut consumption on markers of inflammation and endothelial function: a systematic review and meta-analysis of randomised controlled trials

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The effect of nut consumption on markers of inflammation and endothelial function: a systematic review and meta-analysis of randomised controlled trials

Abstract

Objectives: To examine the effect of nut consumption on inflammatory biomarkers and endothelial function.

Design: A systematic review and meta-analysis

Data sources: Medline, PubMed, CINAHL and Cochrane Central Register of Controlled Trials (all years to 13 January 2017)

Eligibility criteria: Randomised controlled trials (with a duration of three weeks or more) or prospective cohort designs conducted in adults; studies assessing the effect of consumption of tree nuts or peanuts on C-reactive protein (CRP), adiponectin, tumour necrosis factor-alpha, interleukin-6, intercellular adhesion molecule 1, vascular cell adhesion protein 1, and flow mediated dilation (FMD).

Data extraction and analysis: Relevant data was extracted for summary tables and analyses by two independent researchers. Random effects meta-analyses were conducted to explore weighted mean differences (WMD) in change or final mean values for each outcome.

Results: A total of n=32 studies (all randomised controlled trials) were included in the review. The effect of nut consumption on FMD was explored in n=9 strata from n=8 studies (involving n=652 participants), with consumption of nuts resulting in significant improvements in FMD (WMD: 0.79% [95% CI: 0.35, 1.23]). Nut consumption resulted in small, non-significant differences in CRP (WMD: -0.01mg/L [95% CI: -0.06, 0.03]) (n=26 strata from n=25 studies), although sensitivity analyses suggest results for CRP may have been influenced by two

individual studies. Small, non-significant differences were also found for other biomarkers of inflammation.

Conclusions: This systematic review and meta-analysis of the effects of nut consumption on inflammation and endothelial function found evidence for favourable effects on FMD, a measure of endothelial function. Non-significant changes in other biomarkers indicate a lack of consistent evidence for effects of nut consumption on inflammation. The findings of this analysis suggest a need for more research in this area, with a particular focus on randomised controlled trials.

Review registration: CRD42016045424

Strengths and limitations of this study

- This is the first known systematic review and meta-analysis which examined the effect of nut consumption on inflammation and endothelial function, in studies which isolated the effect of nut consumption
- The protocol for the review was pre-registered, and the review followed the requirements of the PRISMA statement
- Risk of bias was assessed using the Cochrane Risk of Bias Tool, and the quality of the body of evidence was then determined using GRADE
- The available evidence base for some of the biomarkers explored was small
- There were variations in the included studies, such as participant health status, nut type and dose, and study duration, although these factors were explored in sub-group analyses

INTRODUCTION

Chronic conditions such as type 2 diabetes, and metabolic syndrome are known to be underpinned by a state of low-grade inflammation, which play a central role in disease progression, and in the development of atherosclerosis[1 2]. Changes in this inflammatory state can be identified via biomarkers of inflammation including C-reactive protein (CRP)[3], tumour necrosis factor-alpha (TNF-α)[4], interleukin-6 (IL-6)[5], and the adhesion molecules intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion protein 1 (VCAM-1)[6], as well as anti-inflammatory biomarkers such as the adipocyte adiponectin[7]. Endothelial dysfunction is a central component in the development and progression of atherosclerosis, with brachial flow mediated dilation (FMD), a non-invasive measure of endothelial function, found to be significantly associated with risk of cardiovascular events[8].

Given that markers of inflammation and endothelial function can indicate changes in disease development and progression, they can be used to explore the impact of consumption of specific foods on health. Nuts contain a wide range of nutrients and bioactive components which may moderate inflammation and the development of endothelial dysfunction, such as alpha-linolenic acid, L-arginine, fibre, and polyphenols[9]. Habitual nut intake has been associated with reduced risk of cardiovascular disease[10], decreased incidence of the metabolic syndrome[11], and decreased risk of diabetes[12]. Clinical trials have previously explored the effects of nut consumption on markers of inflammation and endothelial function, with a range of effects observed[13-22]. A systematic review and meta-analysis would consolidate and appraise the quality of this body of evidence, providing greater clarity where inconsistencies are observed. Even so, the effort is ongoing. For example, a recently published systematic review did not report significant effects of nut consumption on CRP[23], but did not include results of the large PREDIMED study[24]. It is

also possible to consider FMD as an outcome which this previous review did not consider. The aim of the review reported here was to examine the effect of nut consumption on markers of inflammation and endothelial function (CRP, adiponectin, TNF-α, IL-6, ICAM-1, VCAM-1, FMD) in adults. It was hypothesized that the regular inclusion of nuts in a diet would improve markers of inflammation and endothelial function.

METHODS

This systematic review and meta-analysis followed the requirements of the PRISMA statement[25] (Supplementary material 1). The review was registered in PROSPERO, the international prospective register of systematic reviews (http://www.crd.york.ac.uk/PROSPERO; registration number: CRD42016045424).

Study selection

A systematic search of the databases Medline, PubMed, CINAHL and Cochrane Central Register of Controlled Trials was conducted (all years to 13 January 2017). In line with recommendations by Rosen and Suhami[26] both Medline and PubMed were searched to ensure recent studies were detected. Furthermore, where possible, Medical Subject Heading (MeSH) terms as well as free-text search terms were used in the search[26]. Reference lists of eligible articles and relevant reviews were also reviewed for potential studies. An example of the search strategy used is shown in Supplementary material 2. Articles were restricted to those published in English.

To be included in this review, studies were required to meet the following inclusion criteria: 1) randomised controlled trial (including both parallel and cross-over designs) or prospective cohort

design; 2) studies conducted in humans aged 18 years or older; 3) studies assessing the effect of consumption of tree nuts or peanuts on an outcome of interest (CRP, adiponectin, TNF-alpha, IL-6, ICAM-1 VCAM-1, FMD), where the effect of nut consumption could be isolated. The outcomes of interest were selected to cover a suite of biomarkers regularly used in the literature to indicate changes to inflammation and endothelial dysfunction, including in previous meta-analyses exploring the effects of foods and dietary patterns[27 28] ; 4) studies with an intervention duration of three weeks or more (in the case of randomised controlled trials). This minimum duration was selected to ensure included studies reflected sustained changes to inflammation and endothelial function, and to align with similar cut-offs used in other meta-analyses exploring the impact of dietary components on inflammation[27] or the effect of nut consumption on other physiological measures[29 30]. In addition, the following exclusion criteria were applied: 1) studies involving pregnant or breastfeeding women; 2) studies exploring the effects of nut oils or extracts.

Articles were screened based on title and abstract. Full texts were retrieved in the case that an abstract was not available or did not provide sufficient information to draw a conclusion regarding inclusion in the current review. In the case that results from one study were reported in multiple articles, all articles were checked to avoid duplication of study populations in the analysis or overlooking new information on outcomes. Where different information on outcomes were reported across articles, all relevant articles were included in line with the guidelines of the Cochrane Handbook[31]. Where the same outcomes from a single study were reported across multiple articles, decisions relating to article inclusion were based first on the length of follow-up for the outcome, and then by sample size.

Data extraction

The following data were extracted from each study: citation, country, sample size, participant age and body mass index, health status, study design, study duration, nut type, nut dose, details of control arm, and background diet. Mean changes in relevant outcomes were extracted where possible, and in the case that this data was not available, mean final values were retrieved as recommended by the Cochrane Handbook for Systematic Reviews of Interventions[31]. Study authors were contacted for additional details if the published article did not provide sufficient information. Where a study involved more than one intervention group meeting the inclusion criteria, data for the two intervention groups were combined as recommended by the Cochrane Handbook[31]. In the case of the PREDIMED study[24], which included two intervention arms featuring a Mediterranean diet supplemented with either nuts or olive oil, and a low fat control arm, data from the arm receiving the Mediterranean diet with olive oil was treated as the comparator group. This decision was made to ensure outcomes were not confounded by differences in the background diet of the two groups. Where studies reported median rather than mean, medians were used in the meta-analysis, and standard deviation was imputed from interquartile range.

Abstract screening, study inclusion and exclusion, and data extraction were conducted independently by two authors (EN and VG), and any disagreements were resolved via consensus.

Statistical analyses

Review Manager (Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, the Cochrane Collaboration, 2014) was used to conduct random effects meta-analyses to determine the weighted mean differences (WMD) (with 95% confidence intervals) in change or final mean values for each outcome. In initial analyses, cross-over studies

were treated in the same way as parallel studies by comparing measurements from the intervention periods with the control periods via a paired analysis, as the most conservative approach to managing cross-over studies[31]. In order to explore whether this approach affected the final result by under-weighting these studies, paired analyses of cross-over studies using correlation coefficients of 0.25, 0.5, and 0.75 were conducted as sensitivity analyses.

The proportion of total variation attributable to between-study heterogeneity was estimated using the I^2 test statistic[32]. An I^2 value of 75% or greater was deemed to indicate a high level of inconsistency, based on the recommendations by Higgins et al.[32]. I^2 values were generated for each analysis, including sub-group analyses (outlined below). For outcomes with ten or more strata, funnel plots were generated to explore small study effects, with Egger's test used to determine the extent of funnel plot asymmetry[33]. Where funnel plot asymmetry was detected, sensitivity analyses using the trim-and-fill method were conducted to explore potential publication bias[34]. Egger's test and the trim-and-fill method were conducted using Stata (Stata Statistical Software [Computer program]. Release 15. College Station, TX: StataCorp LLC, 2017).In addition to the correlation coefficient sensitivity analyses outlined previously, sensitivity analyses were also conducted to explore the effect of removing studies with imputed standard deviations from analyses, and of removing each individual study in meta-analyses ("leave-one-out" analysis). Pre-specified sub-group analyses were also conducted, based on study duration (less than three months versus more than three months), risk of bias, and nut type. For the purpose of sub-group analyses, studies which compared the effects of two types of nuts to a control[35 36] were classified as 'mixed nut studies'. Post-hoc sub-group analyses were conducted based on health status of participants, whether the energy value of nuts was

substituted for other foods, study design (parallel vs cross-over), and nut dose (<50 grams per day versus ≥ 50 grams per day[29]).

Quality assessment

The Cochrane Collaboration Risk of Bias tool[31] was used to determine the risk of bias in included studies. EN and VG separately appraised the risk of bias and disagreements were resolved by discussion until consensus was reached. The quality of the body of evidence was then determined using GRADE[37], which considers study design, risk of bias, inconsistency, indirectness, imprecision, and other considerations such as publication bias. GRADEproGDT software (GRADEpro. [Computer program on www.gradepro.org]. Version April 2015. McMaster University, 2014) was utilized to conduct the quality of evidence appraisal.

RESULTS

Characteristics of included studies

A total of n=5200 articles were identified from the systematic search and review of relevant reference lists. After applying exclusion criteria, n=36 articles describing n=32 studies (n=34 strata in pooled analyses) were included in the systematic review and meta-analysis. The process of study inclusion and exclusion is shown in Figure 1. Data access is available on request.

Characteristics of included studies are shown in Table 1. All included studies were randomised controlled trials. Although prospective cohort study designs were also considered, no cohort studies met the overall inclusion criteria for the review. The most common reason was that the cohort studies did not report on the association between nut consumption and an outcome of

interest. Fourteen studies had a parallel design[13 15 16 19 35 38-50], 17 had a cross-over design[14 17 18 20-22 36 51-60]. One study[61] combined a parallel and cross-over design, where participants were initially randomised to one of two parallel groups (energy adjusted or ad libitum diet). In this study, each group then took part in the cross-over part of the study consisting of a walnut included period and a walnut excluded period. Amongst all studies, duration ranged from four weeks to five years, although 20[14 15 17 18 21 22 35 36 41 42 47 49 52-56 58-60] out of 32 studies (63%) had a duration of less than three months. Studies were conducted in Spain[16 18 20 36 38 43-47 53], the United States[14 17 22 39 41 48 50 52 54 55 58 59 61], Australia[49 51], India[19 40], Canada[56], South Korea[15], China[21], Brazil[42], South Africa[35], Iran[57], New Zealand[13], and Germany[60]. Studies included participants who were healthy[49 52], had risk factors for chronic disease such as overweight or obesity, dyslipidaemia, hypertension, or pre-diabetes[13 17 18 20 36 40-42 47 50 51 53 55 56 58-60], had type 2 diabetes mellitus[14 21 22 48 57], met the criteria for Metabolic Syndrome[15 16 19 35 38], had diagnosed coronary artery disease[54], or included a mixture of the aforementioned conditions[39 43-46 61]. Included studies examined the effects of consumption of a range of tree nuts including walnuts[17 18 22 39 50 52 53 55 60 61], almonds[21 41 48 54 56 58], pistachios[14 19 20 40 57 59], hazelnuts[13 47], mixed nuts[15 16 38 43-46], and Brazil nuts[49], as well as peanuts[42 51]. In addition, two studies included multiple intervention arms, featuring a different type of nut in each(walnuts and cashews[35], and walnuts and almonds[36]), compared to a control arm. Nuts were consumed in either prescribed doses, ranging from approximately 18[49] to 85 grams per day[54], or were designed to provide a set proportion of dietary energy, so the amount would vary for individuals[14 18 19 21 35 50 58 59]. Background diets consisted of either participant's habitual diet, or a prescribed diet aligned with healthy lifestyles such as the NCEP Step I or II diet, a Mediterranean-style diet, the Therapeutic Lifestyle Changes diet or another prudent style diet in line with dietary guidelines. Six studies provided all or the majority of foods under

controlled feeding conditions[14 21 35 55 58 59]. Twenty-two studies[14 17-22 35 36 39 40 42-47 50 53-56 58-60] prescribed diets accounting for the energy value of the nuts, either quantitatively through dietary modelling (including the energy value of the nuts within the total energy value of the diet) or qualitatively by encouraging participants to substitute nuts for items with similar energy values. One study[61] included an intervention group where participants were advised on food substitutions to account for the energy value of the provided nuts, and another intervention group where energy intake was not prescribed (ad libitum food consumption). During the control diets or periods, participants typically consumed a similar diet but without nuts, although some studies included control diets with a specific product substituted for the nuts, such as eggs[52], olive oil[36 43-46], muffins[56], and chocolate[41], amongst others. Only two studies[42 50] stated they prescribed a set energy restriction for both intervention and control groups; all other studies utilised isocaloric diets for weight maintenance or ad libitum diets. No studies reported a significant difference in weight loss between the intervention and control groups.

Table 1: Characteristics of included randomised controlled trials examining the effect of nut consumption on inflammatory biomarkers and endothelial function

Citation and country	Sample size (for analysis)	Mean age, years	Mean BMI, kg/m^2	Population	Design	Study duration, weeks	Nut type	Nut dose	Comparison group details	Background diet	Outcome of interest
Barbour et al. (2015)[51], Australia	61 (M: 29, F: 32)	65 ± 7	31 ± 4	Overweight	X	12	Peanut (high oleic)	M: 84g, 6 x week F: 56g, 6 x week	No nuts	Habitual diet	CRP (mg/L)
Burns-Whitmore et al. (2014)[52], United States	20 (M: 4, F: 16)	38 ± 3	23 ± 1	Healthy	X	8	Walnut	28.4g, 6 x week	Standard egg, 6x week*	Habitual diet	CRP (ng/mL)‡‡‡, TNF-α (pg/mL), IL-6 (pg/mL), ICAM-1 (ng/mL)
Canales et al. (2011)[53], Spain	22 (M: 12, F: 10)	54.8 (SEM: 2.0)	29.6 (SEM: 0.7)	Overweight with at least one risk factor for CVD	X	5	Walnut	150g/week walnut paste integrated into steaks and sausages	Low-fat steaks and sausages	Habitual diet with substituted meat products	ICAM-1 (µg/L)‡‡‡, VCAM-1 (µg/L)‡‡‡
Casas-Agustench et al. (2011)[16], Lopez-Uriarte et al. (2010)[38], Spain	50 (M: 28, F: 22)	*I:* 52.9 ± 8.4 *C:* 50.6 ± 8.4	*I:* 31.6 ± 2.8 *C:* 30.0 ± 3.3	MetS	P	12	Mixed nuts (walnut, almond, hazelnut)	30g/day (15g walnuts, 7.5g almonds, 7.5g hazelnuts)	No nuts	American Heart Association dietary guidelines	CRP (mg/L), adiponectin (ng/mL)‡‡‡, IL-6 (ng/L)‡‡‡, ICAM-1 (µg/L)‡‡‡, VCAM-1 (µg/L)‡‡‡
Chen et al. (2015)[54], United States	45 (M: 18, F: 27)	61.8 ± 8.6	30.2 ± 5.1	CAD	X	6	Almond	85g/day	No nuts	NCEP Step 1 diet (isocaloric)	CRP (mg/L), TNF-α (pg/mL), IL-6 (pg/mL),

											VCAM-1 (ng/mL), FMD (%)
Chiang et al. (2012)[55], United States	25 (M: 14, F: 11)	33 (range 23 - 65)	24.8 (range: 18.7 - 36.6)	Normal to HL	X	4	Walnut	42.5g per 10.1MJ (6 x week)	No nuts or fatty fish*	American Dietary Guidelines (isocaloric)	CRP (mg/L)***, TNF-α (pg/mL), IL-6 (pg/mL), ICAM-1 (ng/mL)
Damasceno et al. (2011)[36], Spain	18 (M: 9, F: 9)	56 ± 13	25.7 ± 2.3	HC	X	4	1.Walnut 2. Almond	1. 40 - 65g/day walnuts 2. 50 - 75g/day almonds§§§	35 – 50g/day virgin olive oil	Mediterranean -style diet (isocaloric)	CRP (mg/L), ICAM-1 (ng/mL), VCAM-1 (ng/mL)
Djousse et al. (2016)[39], United States	26 (M: 10, F: 16)**	*I:* 60.8 ± 11.3 *C:* 68.8 ± 10.9	*I:* 29.6 ± 5.2 *C*: 33.5 ± 8.7	CAD or T2DM	P	12	Walnut	28g/day	No nuts	Habitual diet with walnuts substituted for equivalent kJ items	Adiponectin (µg/mL)
Gulati et al. (2014)[19], India	68 (M: 37, F: 31)	42.5 ± 8.2	30.9 ± 7.5	MetS	P	24	Pistachio	20% of total energy•••	Dietary guidelines for Asian Indians	Dietary guidelines for Asian Indians, with pistachios substituted for diet components	CRP (mg/L)***, adiponectin (µg/mL)***, TNF-α (pg/mL)
Hernandez-Alonso et al. (2014)[20], Spain	54 (M: 29, F: 25)	55 (95% CI: 53.4, 56.8)	28.9 (95% CI: 28.2, 29.6)	Pre-diabetic	X	16	Pistachio	57g/day	Intake of fatty foods adjusted to account for energy from pistachios	Isocaloric diet	Adiponectin (µg/mL)***, IL-6 (pg/mL)
Hu et al. (2016)[49], Australia	21 (M, F)‡‡	*I*: 62.4 ± 8.8 *C*: 66.5 ± 6.9	*I*: 82.2 ± 10.8 *C*: 83.9 ± 22.4§§	Healthy	P	6	Brazil nut (plus green tea extract)	18g/day¶¶	Green tea extract, no nuts	Habitual diet	CRP (mg/L)
Jenkins et al. (2002)[56], Canada	27 (M: 15, F: 12)	64 ± 9	25.7 ± 3.0	HL	X	4	Almond	73 ± 3 g/day¶¶	147 ± 6 g/day muffins¶¶,*	NCEP Step 2 diet (isocaloric)	CRP (mg/L)
Kasliwal et al.	56 (M: 46,	39.3 ±	*I:* 26.1 ±	DL	P	12	Pistachio	40g/day	No nuts	Therapeutic	CRP

(2015)[40], India	F:10) (randomised) 42 (completed)	8.1††	2.9†† *C:* 27.8 ± 4.7††					shelled		Lifestyle Change diet	(mg/L), FMD (%)
Katz et al. (2012)[17], United States	46 (M: 18, F: 28)	57.4 ± 11.9	33.2 ± 4.4	Overweight plus risk factors for MetS	X	8	Walnut	56g/day	No nuts	Ad libitum, participants advised to substitute walnuts for other foods	FMD (%)
Kurlansky and Stote (2006)[41], United States	47 (F)	*Almond:* 41.8 ± 11.7 *Almond + chocolate:* 46.2 ± 7.8 *Chocolate :* 36.5 ± 11.9 *C:* 51.3 ± 6.3	*Almond:* 25.3 ± 3.5 *Almond + chocolate:* 27.2 ± 4.2 *Chocolate:* 23.9 ± 3.3 *C:* 26.1 ± 4.1	Healthy, including HC	P	6	Almond	1. 60g/day 2. 60g almonds/ day + 41g dark chocolate/ day	1. 41g dark chocolate/day 2. self-selected diet	Therapeutic Lifestyle Change diet (isocaloric)	CRP (mg/L), ICAM-1 (ng/mL), VCAM-1 (ng/mL)
Lee et al. (2014)[15], South Korea	60 (M, F)‡‡	ages 35 - 65 eligible for study	*I:* 27.19 ± 2.11 *C:* 26.96 ± 2.16	MetS	P	6	Mixed nuts (walnut, pine nut, peanut)	30g mixed nuts/day (15g walnuts, 7.5g pine nuts, 7.5g peanuts)	Prudent diet	Prudent diet (isocaloric)	CRP (mg/L), adiponectin (µg/mL), IL-6 (pg/mL), ICAM-1 (ng/mL), VCAM-1 (ng/mL)
Liu et al. (2013)[21], China	20 (M: 9, F: 11)	58 ± 2	26.0 ± 0.7	T2DM and HL	X	4	Almond	56g/day¶¶ (20% energy)	NCEP Step II diet	NCEP Step II diet (isocaloric diet)	CRP (mg/L), TNF-α (ng/L)‡‡‡, IL-6 (ng/L)‡‡‡, ICAM-1 (µg/L)‡‡‡, VCAM-1 (µg/L)‡‡‡
Ma et al. (2010)[22],	24 (M: 10, F: 14)	58.1 ± 9.2	32.5 ± 5.0	T2DM	X	8	Walnut	56g/day	No nuts	Ad libitum, participants	FMD (%)

United States										advised to substitute walnuts for other foods	
Moreira Alves et al. (2014)[42], Brazil	65 (M)	*High oleic peanuts:* 27.2 ± 6.1 *Peanuts:* 27.6 ± 1.5 *C:* 27.1 ± 1.6	29.8 ± 2.3	Overweight	P	4	Peanut (high oleic and con-ventional)	1. 56g/day high oleic peanuts 2. 56g/day conventio nal peanuts	No peanuts	Hypocaloric diet (250 kcal/day deficit)	CRP (mg/L)***, TNF-α (pg/mL)
Mukuddem-Petersen et al. (2007)[35], South Africa	64 (M: 29, F: 35)	45 ± 10	*Walnut:* 36 (95% CI: 33.3 - 38.7) *Cashew:* 34.4 (95% CI: 32.3 - 36.6) *C:* 35.1 (95% CI: 32.8 - 37.4)	MetS	P	8	1. Walnut 2. Cashew	1. 20% energy from walnuts 2. 20% energy from cashews§ §§	No nuts	Controlled feeding protocol (isocaloric)	CRP (mg/L)
Njike et al. (2015)[61], United States	112 (M: 31, F: 81)	*Ad libitum:* 56.5 ± 11.7 *Energy adjusted:* 53.3 ± 11.1	*Ad libitum:* 30.0 ± 4.0: *Energy adjusted:* 30.2 ± 4.1	Overweight, pre-diabetic or MetS	X••	24	Walnut	56g/day	No nuts	1. Ad libitum diet 2. Isocaloric diet (energy adjusted for walnuts)	FMD (%)
Parham et al. (2014)[57], Iran	44 (M: 11, F: 33)	*Interventi on first:* 53 ± 10 *Control first:* 50 ± 11	*Intervention first:* 32.16 ± 6.58 *Control first:* 30.24 ± 4.03	T2DM	X	12	Pistachio	50g/day	No pistachios	Ad libitum	CRP (mg/dL)‡‡‡
PREDIMED (Casas et al., 2014[43], Casas et al., 2016[44], Lasa et al., 2014[45], Urpi-Sarda et al., 2012[46]), Spain	353 (M: 172, F: 181)‡ 124 (M: 45, F: 79)• 110 (M: 55, F: 55)§ 108 (M: 54, F: 54)¶	Range: 55 – 80 (M), 60 – 80 (F)	29.4 ± 3.4‡	T2DM and/or CHD risk factors	P	52 ‡,•,§ 260 (5 years)¶	Mixed nuts (walnut, almond, hazelnut)	30g/day (15g walnuts, 7.5g hazelnuts, 7.5g almonds)	1L olive oil per week†	Mediterranean diet	CRP (mg/L) †††, adiponectin (µg/mL), TNF-α (pg/mL), IL-6 (pg/mL), ICAM-1 (µg/L)‡‡‡,

											VCAM-1 (ng/mL)
Rajaram et al. (2010)[58], United States	25 (M: 14, F: 11)	41 (SEM: 13)	71 (SEM: 2.7)§§	Healthy (including overweight) to HC	X	4	Almond	1. 10% energy 2. 20% energy§§§	No nuts	Cholesterol lowering diet (isocaloric)	CRP (mg/L), IL-6 (ng/L)‡‡‡
Rock et al. (2016)[50], United States	126 (F)	50 (range: 22 - 72)††	33.5 (range: 27 - 40)††	Overweight	P	52	Walnut	42g/day¶¶ (18% energy)	1. higher fat (35% energy) lower CHO (45% energy) diet, no nuts*	Hypocaloric diet (500 - 1000 kcal/day deficit)	CRP (ug/mL)‡‡‡, IL-6 (pg/mL)
Ros et al. (2004)[18], Spain	20 (M: 8, F: 12)	55 (range: 26 - 75)	70.6 ± 10.3§§	HC	X	4	Walnut	40 – 65g/day (~18% energy) §§§	No nuts	cholesterol lowering Mediterranean diet (isocaloric)	CRP (mg/L)***, ICAM-1 (µg/L)‡‡‡, VCAM-1 (µg/L)‡‡‡, FMD (%)
Sauder et al. (2015)[14], United States	30 (M: 15, F: 15)	56.1 ± 7.8	31.2 ± 3.1	T2DM	X	4	Pistachio	20% total energy§§§	Therapeutic Lifestyle Changes diet	Therapeutic Lifestyle Changes diet (isocaloric)	CRP (mg/L), ICAM-1 (ng/mL), VCAM-1 (ng/mL), FMD (%)
Sola et al. (2012)[47], Spain	56 (M: 23, F: 33)	*I:* 56.79 ± 10.46 *C:* 49.79 ± 9.53	*I:* 27.30 ± 3.01 *C:* 28.31 ± 3.25	Pre-HT or HT with at least one risk factor for CVD	P	4	Hazelnut	30g/day (in cocoa cream product)	Cocoa cream product*	Low saturated fat diet (isocaloric)	CRP (mg/L), IL-6 (pg/mL), ICAM-1 (ng/mL), VCAM-1 (ng/mL)
Sweazea et al. (2014)[48], United States	21 (M: 9, F: 12)	*I:* 57.8 ± 5.6 *C:* 54.7 ± 8.9	*I:* 37.2 ± 7.8 *C:* 33.5 ± 8.8	T2DM	P	12	Almond	43g (5-7 x week)	≤ 2 servings non-trial nuts/week	Habitual diet	CRP (mg/L), TNF-α (pg/mL), IL-6 (pg/mL)
Tey et al. (2014)[13], New Zealand	107 (M: 46, F: 61)	42.5 ± 12.4	30.6 ± 5.1	Overweight	P	12	Hazelnut	1. 30g/day 2. 60g/day	No nuts	Habitual diet	CRP (mg/L), IL-6 (pg/mL), ICAM-1 (µg/L)‡‡‡, VCAM-1

											(µg/L)‡‡‡
West et al. (2012)[59], United States	28 (M: 10, F: 18)	48 (SEM: 1.5)	26.8 (SEM: 0.7)	HL	X	4	Pistachio	1. 10% energy 2. 20% energy§§§	NCEP Step 1 diet	Isocaloric diet	FMD (%)
Wu et al. (2014)[60], Germany	40 (M: 10, F: 30)	60 ± 1	24.9 ± 0.6	Healthy (including overweight)	X	8	Walnut	43g/day	No nuts	Western diet with walnuts substituted for saturated fat (isocaloric)	CRP (mg/dL)‡‡‡, adiponectin (µg/mL)***, ICAM-1 (ng/mL), VCAM-1 (ng/mL)

*Study included other intervention group which was not relevant to this review, therefore this group was not included in this analysis
†Treated as comparison group for this analysis
‡ICAM [46]
•Adiponectin [45]
§VCAM-1 [43]
¶CRP, IL-6, TNF-α [44]
**Gender breakdown estimated from % males reported in paper
††Characteristics reported for randomised participants
‡‡Gender breakdown for analysed participants not available
••Participants were randomised to one of two parallel groups (ad libitum or calorie adjusted). Within each group participants completed a 'walnut included' and 'walnut excluded' period in a cross-over design
§§ Body weight (kg) is reported when BMI was not available
¶¶ Mean intake
•••Dose based on reference individual listed in Gulati et al. [19]
§§§Gram weight for dose sub-analysis based on mid-point of range of doses used
***Units confirmed with study authors
††† Units based on primary publication[62]
‡‡‡Unit reported in study, converted to consistent unit for analysis
Abbreviations: BMI: body mass index; CAD: coronary artery disease; CHD: coronary heart disease; CI: confidence intervals; CVD: cardiovascular disease; DL: dyslipidaemia; F: female; HL: hyperlipidaemia; HT: hypertension; M: male; MetS: metabolic syndrome; NCEP: National Cholesterol Education Program; P: parallel; SEM: standard error of mean; T2DM: type 2 diabetes mellitus; X: cross-over

Effect of nut consumption on study outcomes

FMD

A total of nine strata from eight studies[14 17 18 22 40 54 59 61] explored the effect of nut consumption on FMD. Of the nine strata, five explored the effect of walnut consumption on FMD[17 18 22 61], and six had a duration of less than three months[14 17 18 22 54 59]. The meta- analysis showed that nut consumption was associated with a significant increase in FMD (Figure 2 and Table 2). Sensitivity analyses indicated that excluding any one study did not substantially alter the effect (data not shown). The effect estimate was also similar after using different correlation coefficients (CC: 0.5, Supplementary material 3; CC: 0.25 and 0.75, data not shown). No significant differences were found for sub-group analyses (Supplementary material 4) although it was noted that when sub-group comparisons were made according to nut type, only the walnut sub-group found significant improvements in FMD.

CRP

A total of 26 strata from 25 studies[13-16 18 19 21 35 36 40-42 44 47-52 54-58 60] explored the effect of nut consumption on CRP. Almonds were the most common nut type used in these analyses (seven strata[21 41 48 54 56 58]), followed by walnuts[18 50 52 55 60] and mixtures of more than one nut type[15 16 35 36 44] (each used in five strata). A total of 17 strata from 16 studies had a duration of less than three months[14 15 18 21 35 36 41 42 47 49 52 54-56 58 60]. When all studies were included in the meta-analysis, nut consumption resulted in non-significant differences in CRP (Figure 3 and Table 2). The overall effect was relatively unchanged when studies with imputed standard deviations were removed from the analysis (Table 2). Sensitivity analyses identified two studies[15 52] that contributed substantially to the pooled result, as when they were excluded from the meta-

analysis, the reductions in CRP were significant (Supplementary material 5). In addition, the use of different correlation coefficients did not change the overall effect found (CC: 0.5, Supplementary material 3; CC: 0.25 and 0.75, data not shown). Sub-group analyses indicated that statistically significant differences were found between studies which included the energy value of nuts in the prescribed diet compared to those that did not (Supplementary material 4). An effect estimate of -0.23 mg/L [-0.44, -0.01] was found for studies in which diets incorporated the energy value of nuts, whilst an effect estimate of -0.00 mg/L [-0.06, 0.05]) was found for studies which did not ($Chi^2 = 3.99$, df = 1 (P = 0.05), $I^2 = 74.9\%$). When studies were grouped according to nut dose, an effect estimate of -0.00 mg/L [0.00, 0.00] was found for studies which included less than 50 grams of nuts/day, whilst an effect estimate of -0.34 mg/L [-0.63, -0.06]) was found when 50 grams or more were used ($Chi^2 = 5.74$, df = 1 (P = 0.02), $I^2 = 82.6\%$). Borderline significant differences (p=0.05) were found when studies with a parallel design were compared to cross-over studies. However, when either of the studies identified in the sensitivity analysis[52,15] were excluded, these sub-group analyses no longer produced significant results (data not shown).

Adiponectin, TNF-α, IL-6, ICAM-1, VCAM-1

The meta- analysis showed that consumption of nuts did not result in significant differences in adiponectin, TNF-α, IL-6, ICAM-1, or VCAM-1 (Table 2 and Supplementary material 6). In the case that pooled analyses featured studies with imputed standard deviations (IL-6, ICAM-1, VCAM-1), excluding these studies did not substantially change the effect estimates (Table 2). Sensitivity analyses indicated that excluding any one study did not substantially alter the effect (data not shown). Overall effects also did not change when different correlation coefficients were used for cross-over studies (CC: 0.5, Supplementary material 3; CC: 0.25 and 0.75, data not

shown). No significant differences between sub-groups were observed (Supplementary material 4).

Table 2: Differences in FMD, CRP, adiponectin, TNF-α, IL-6, ICAM-1, and VCAM-1following nut consumption, compared to control.

Outcome	Analysis description	Number of studies	Number of strata	Number of participants	Effect estimate		Inconsistency (I^2)
FMD (%)	**All studies‡**	8	9	652	0.79% [0.35, 1.23], P<0.001	-0.40% [-1.72, 0.92] - 2.36% [-1.71, 6.43]	0%
CRP (mg/L)	**All studies**	25	26	1578	-0.01mg/L [-0.06, 0.03], P = 0.59†	-5.53mg/L [-11.96, 0.90] - 0.60mg/L [-2.44, 3.64]	20%
	Imputed SD excluded*	19	20	1244	-0.01mg/L [-0.06, 0.04], P = 0.71	-5.53mg/L [-11.96, 0.90] - 0.60mg/L [-2.44, 3.64]	26%
Total adiponectin (µg/mL)	**All studies‡**	7	7	506	0.29 µg/mL [-0.63, 1.21], P = 0.53	-9.80µg/mL [-23.99, 4.39] - 10.60µg/mL [6.39, 14.81]	79%
TNF-α (pg/mL)	**All studies‡**	8	8	482	-0.05 pg/mL [-0.13, 0.02], P = 0.17	-3.70pg/mL [-6.93, -0.47] - 0.70pg/mL [-0.41, 1.81]	2%

IL-6 (pg/mL)	**All studies**	13	13	906	-0.02 pg/mL [-0.12, 0.08], P = 0.65,	-1.55pg/mL [-2.80, -0.30] - 0.46pg/mL [-0.22, 1.14]	10%
	Imputed SD excluded	11	11	800	-0.09 pg/mL [-0.23, 0.05], P = 0.19	-0.50pg/mL [-1.62, 0.62] - 0.46pg/mL [-0.22, 1.14]	0%
ICAM-1 (ng/mL)	**All studies**	14	15	1047	0.68 ng/mL [-0.53, 1.89], P = 0.27	-80.63ng/mL [-209.62, 48.36] - 16.76ng/mL [1.44, 32.08]	0%
	Imputed SD excluded	13	14	1011	0.68 ng/mL [-0.53, 1.89], P = 0.27	-80.63ng/mL [-209.62, 48.36] - 16.76ng/mL [1.44, 32.08]	0%
VCAM-1 (ng/mL)	**All studies**	13	14	804	2.83 ng/mL [-8.85, 14.51], P = 0.63	-99.72ng/mL [-316.35, 116.91] - 62.00ng/mL [-80.23, 204.23]	0%
	Imputed SD excluded	12	13	768	2.43 ng/mL [-9.29, 14.15], P = 0.68	-99.72ng/mL [-316.35, 116.91] - 46.34ng/mL [-	0%

						22.06, 114.75]	

*Sensitivity analysis where studies with an imputed standard deviation were excluded

†Sensitivity analyses indicated that exclusion of either of two studies[15 52] resulted in an effect estimate of -0.22 [-0.40, -0.04].

‡No studies reporting FMD, adiponectin or TNF-α, required imputation of standard deviation

Small study effects

Funnel plots were generated for outcomes with ten or more strata (CRP, IL-6, ICAM-1, and VCAM-1) (Supplementary material 7). Egger's test indicated asymmetry in funnel plots for CRP (bias = -0.68 [95% CI = -1.06 to -0.31], P = 0.001) and IL-6 (bias = -0.81 [95% CI = -1.45 to -0.16], P = 0.02), suggesting the presence of small study effects which may have been attributable to publication bias. Use of the trim-and-fill method did not change these results (data not shown). Funnel plot asymmetry was not detected for ICAM-1 or VCAM-1 (data not shown).

Risk of bias and quality of the body of evidence

The risk of bias was determined for each strata using the Cochrane Risk of Bias Tool and the results of the assessment are shown in Figure 4 and Supplementary materials 8 and 9. The quality of the evidence was 'high' for FMD, ICAM-1, and VCAM-1. The quality was downgraded to 'moderate' for TNF-α due to risk of bias, and to 'low' for CRP and IL-6 due to both risk of bias and the possibility of publication bias. The quality of the evidence for adiponectin was downgraded to 'very low' due to risk of bias, inconsistency, and imprecision (Supplementary material 10).

DISCUSSION

The results of this systematic review and meta-analysis suggested favourable effects of nut consumption on FMD, a measure of endothelial function. These findings align with a review conducted in 2011 by the European Food Safety Authority (EFSA), which explored the effects of walnut consumption on endothelium-dependent vasodilation [63]. A meta-analysis was not part of the EFSA report[63], but the present study provides a meta-analysis that includes more recently

published research[17 61]. It also includes studies investigating other types of nuts[14 40 54 59]. Sub-group analyses found significant improvements in FMD only in those studies using walnuts, consistent with the EFSA report which only examined walnut consumption, although the test for sub-group differences in the present study did not reach statistical significance. This may have resulted from the small number of studies available for assessing FMD. Having few studies may have also played a role in the lack of significant effects observed in other FMD sub-group analyses. These include studies in participants with type 2 diabetes, or studies lasting longer than three months. Further research is therefore required in this area.

Despite the small sample size, the findings of this review relating to FMD are of value due to the known associations between FMD and future cardiovascular events. A meta-analysis of cohort studies found a significant reduction in risk of cardiovascular events per 1% increase in FMD (RR: 0.872 [95% CI: 0.832 – 0.914])[8]. In comparison, the present study found an effect estimate of 0.79% for nut consumption compared to controls, suggesting these results are likely to be of clinical relevance to future cardiovascular risk. There are a number of mechanisms by which nuts, and walnuts in particular, could improve FMD. FMD is a measure of endothelial dysfunction[64], a condition characterised by reduced availability of the vasodilator nitric oxide (NO)[65]. Nuts contain high levels of L-arginine[66], an amino acid which acts as a precursor to NO[67]. Walnuts in particular are rich in alpha-linolenic acid, a polyunsaturated fatty acid that has been suggested to increase membrane fluidity, thus also increasing nitric oxide synthesis and release[68]. The antioxidant content of nuts may also play a role in the improvements in endothelial function observed[9].

Our finding of no significant effects on inflammatory biomarkers CRP, TNF-α, IL-6, ICAM-1, VCAM-1, or the anti-inflammatory biomarker adiponectin reflects the body of evidence

available at this time. There may be effects with CRP but characteristics of the study sample or design of the dietary intervention may influence the ability to detect these effects. Sensitivity analyses indicated that results may have been disproportionally influenced by a small number of studies. Exclusion of either one of two studies[15 52] resulted in the meta-analysis yielding significant reductions in CRP following nut intake, suggesting these two studies were responsible for the results found. This appears to be the result of low reported CRP values and correspondingly small standard errors, resulting in these studies receiving substantially higher weighting than other studies in the pooled analysis. The study sample may in part explain these findings, as the study by Burns-Whitmore et al.[52] was conducted in healthy lacto-ovo vegetarians. Consumption of a plant-based diet has been associated with decreased inflammation[69]. In contrast, Lee et al.[15] explored the effect of nut consumption in individuals with Metabolic Syndrome, which is typically associated with elevated CRP levels[70]. Reported units were confirmed with study authors.

The findings of this review may also have been influenced by the design of the dietary interventions included. Sub-group analyses found significant reductions in CRP when studies incorporated 50 grams or more of nuts per day. This finding aligns with previous research suggesting a dose-response effect of nut intake on other outcomes such as cholesterol[71]. However, these findings should be interpreted with caution, as several studies[14 18 19 21 35 50 58 59] incorporated nuts as a proportion of total energy, resulting in substantial variation between individuals in the dose consumed. Furthermore, whether the energy value of nuts was adjusted for in the total diet may have influenced results. Sub-group analyses suggested significant effects on CRP were only found when the energy provided by nuts was accounted for either by dietary modelling or advice to substitute other foods for nuts. This aligns with a previous review by our

group which highlighted the importance of considering total energy intake in trials examining the effect of vegetable intake on weight loss[72]. There is also evidence to suggest markers of inflammation such as CRP may be reduced following periods of energy restriction[73], highlighting the importance of considering total energy intake when exploring the effects of individual foods. The design of the control arm may have also impacted on results, as several studies[36 43-46] compared intake of nuts to a control intervention which also had the potential to influence inflammation and endothelial function, for example olive oil[27]. The potential impact of control groups on underestimating intervention effects has previously been highlighted in the weight loss literature[74]. Trials aiming to explore the influence of specific foods on health outcomes must carefully consider the design of the dietary intervention and control arms, and aim to avoid increases in total energy intake which could skew results.

The heterogeneity in study design elements, particularly related to dietary intervention, may explain why reviews exploring the effects of nut consumption on inflammation have found varying results. Although including fewer studies than in our review, a recently published review by Mazidi et al.[23] also found non-significant differences in inflammatory biomarkers (CRP, IL-6, adiponectin, ICAM-1, and VCAM-1), although in contrast to our review they observed a small increase in CRP levels. The review by Mazidi et al.[23] appeared to have broader eligibility criteria which also included post-prandial studies and those exploring the effects of soy consumption. In another review Barbour et al.[75] reported significant reductions in CRP following nut consumption. It should be noted however, that Barbour et al.[75] included studies where nut consumption was encouraged as part of a suite of favourable dietary changes not matched in control groups, meaning the effect of the nuts themselves could not be isolated. In these circumstances it may not be possible to show whether effects observed were the result of

increases in nut intake, or the wider dietary changes occurring. We avoided this problem by excluding studies with a portfolio of dietary changes not matched in the control group, or by treating a comparable intervention group as the "control" (or comparator), as in the case of the PREDIMED study[24]. Nevertheless, nuts appear in healthy dietary patterns and we have previously shown that consumption of a healthy dietary pattern (many of which include habitual nut intake) results in significant reductions in CRP[76].

It should be noted that while the current analysis found favourable effects of nut consumption on a marker of endothelial dysfunction, the lack of evidence for effects on cell adhesion molecules VCAM-1 and ICAM-1 suggests changes in endothelial cell activation may not have occurred. Given that the inflammatory cytokines which characteristically induce endothelial cell activation (for example TNF-α and IL-6)[65] also appeared unchanged, the lack of difference found for ICAM-1 and VCAM-1 is perhaps not surprising. More research on this cluster of molecules will be informative.

This review had a number of strengths. It used a systematic methodology following current guidelines for systematic reviews, including prospective registration, and used the Cochrane Risk of Bias tool and GRADE method to evaluate the quality of evidence. We considered a range of biomarkers associated with inflammation and endothelial function, including the anti-inflammatory adipocyte adiponectin. These biomarkers were selected to reflect changes in disease progression and amelioration, in order to explore mechanisms responsible for the favourable effects of nut consumption on cardiovascular disease[10] and other chronic conditions[11,12]. However we fully acknowledge that the measures explored here are not interchangeable with disease endpoints such as mortality and morbidity. The size of the evidence base, including the small number of participants available for analyses of individual biomarkers, is a limitation,

particularly with respect to generalisability and strength of the evidence. Furthermore, although we were unable to explore the distribution of the published data included in this meta-analysis, the fact that several studies reported median values rather than means suggests some of the data may have been skewed, which may have impacted upon our analyses.

The heterogeneity of the evidence base included can be also considered a limitation of this review. Variation existed as a result of participant health status, nut type and dose, and study duration, although these factors were explored in sub-group analyses. Statistically significant sub-group differences were found only for CRP when studies were grouped according to whether they incorporated the energy value of nuts into the diet, and based on nut dose (<50 grams/day versus ≥50 grams/day). However due to the small number of studies, it is possible that other sub-group differences may have been found if the sample size was larger. For example, borderline significant differences (p=0.05) were found between the study designs, with larger reductions in CRP found for cross-over design studies. As the nature of cross-over studies eliminates between-subject variation[77], they may provide superior insights when exploring the impact of dietary interventions on biomarkers such as CRP, however their results may also be impacted by carry-over effects[31]. Given the short or absent wash-out periods of some of the included studies[18 36 51 55 58], the potential impact of carry-over effects cannot be ruled out. Background diets also varied between studies, with some studies prescribing diets based on dietary guidelines, whereas others allowed participants to follow their habitual diet, which may have varied substantially between individuals. Analysis of funnel plots suggested the results for CRP and IL-6 may have been influenced by small study effects (which could indicate publication bias), which resulted in downgrading the quality of the evidence for these outcomes. Funnel plot asymmetry remained after sensitivity analyses were conducted. These findings suggest the need for more research in

this area, with a particular focus on the registration of study protocols with detailed information on primary and secondary outcomes, to reduce the potential for publication bias.

This systematic review and meta-analysis of the effects of nut consumption on inflammation and endothelial function found evidence for favourable effects on FMD, a measure of endothelial function. Non-significant differences in CRP, adiponectin, TNF-α, IL-6, ICAM-1, VCAM-1 suggest a lack of consistent available evidence for effects of nut consumption on inflammation, although the results for CRP should be interpreted with caution due to the large influence of single studies on the pooled results. The findings of this review provide further insight into the mechanisms by which nut consumption may exert favourable effects on the risk of chronic conditions such as cardiovascular disease. The findings also build on previous research such as the 2011 EFSA report[63] on walnut consumption and endothelial-dependent vasodilation, and reinforce the value of including nuts within a healthy dietary pattern. However, the small evidence base for FMD and the observed lack of consistency in findings relating to inflammation suggest a need for more research in this area, with a particular focus on randomised controlled trials incorporating the energy value of nuts into the total diet. There is also a need for the transparent registration of trial protocols, as well as appropriate dietary controls. These could include healthy dietary patterns (not including nuts), with a greater emphasis on dietary modelling required to ensure nutrient intakes are matched between control and intervention groups, minimising the risk of confounding.

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Data sharing statement:

Access to data available on request (elizan@uow.edu.au)

Author contributions:

Study concept and design: Neale, Tapsell, Batterham

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Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Neale, Guan, Batterham

Obtained funding: Tapsell, Neale, Batterham

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Study supervision: Tapsell, Batterham

Conflict of Interest Disclosures:

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Figure titles:

Figure 1: PRISMA[25] flow diagram of study selection

Figure 2: Difference in FMD (%) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals.

Figure 3: Difference in C-reactive protein (mg/L) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals.

Figure 4: Risk of bias assessment as proportion of total strata.

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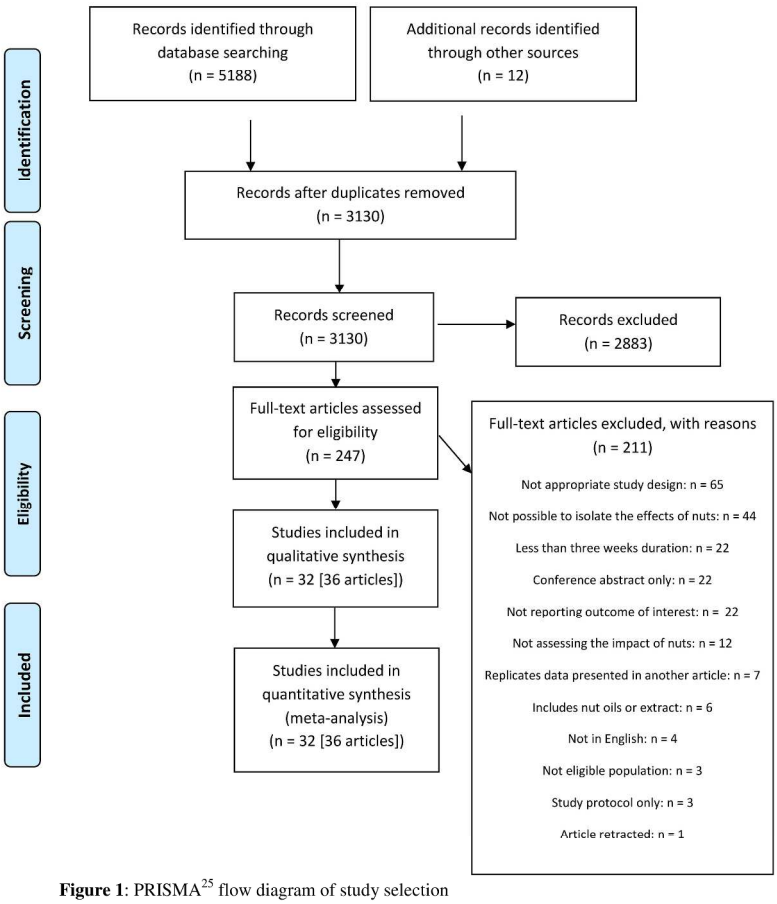

Figure 1: PRISMA[25] flow diagram of study selection

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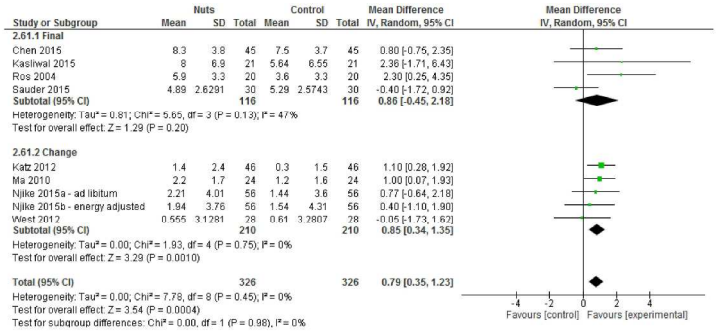

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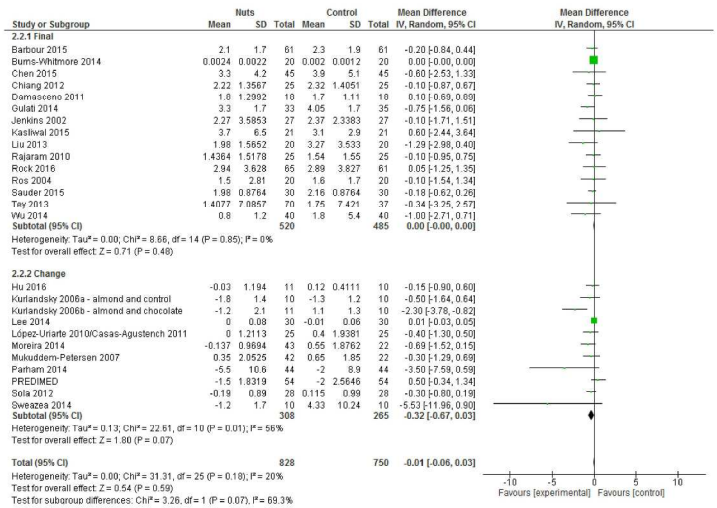

Figure 3: Difference in C-reactive protein (mg/L) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals.

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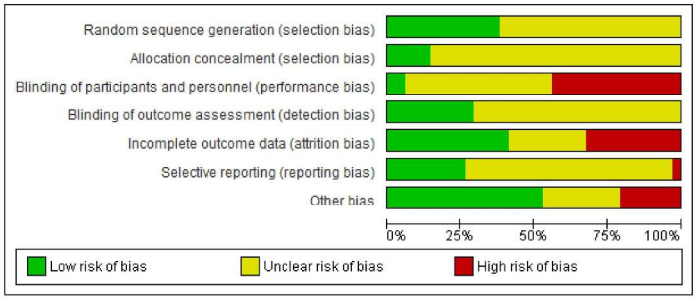

Figure 4: Risk of bias assessment as proportion of total strata.

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279x361mm (300 x 300 DPI)

List of supplementary material

Supplementary material 1: PRISMA checklist (as separate file)

Supplementary material 2: Example search strategy

Supplementary material 3: Differences in CRP, adiponectin, TNF-α, IL-6, ICAM-1, VCAM-1, and FMD following nut consumption, compared to control, using correlation coefficient of 0.5

Supplementary material 4: Results of sub-group analyses

Supplementary material 5: Forest plots of difference in CRP after exclusion of individual studies

Supplementary material 6: Forest plots of differences in biomarkers between nut consumption and control

Supplementary material 7: Funnel plots

Supplementary material 8: Risk of bias assessment summary

Supplementary material 9: Justification for risk of bias judgements

Supplementary material 10: GRADE assessment of the quality of the body of evidence

Supplementary material 2:

Search strategy: PubMed

((((((((((((((((((((("nuts"[MeSH Terms]) OR nut) OR nuts) OR "juglans"[MeSH Terms]) OR walnut*) OR "prunus dulcis"[MeSH Terms]) OR almond*) OR "bertholletia"[MeSH Terms]) OR brazil nut*) OR Amazonia) OR "anacardium"[MeSH Terms]) OR cashew*) OR "corylus"[MeSH Terms]) OR hazelnut*) OR "macadamia"[MeSH Terms]) OR macadamia*) OR "carya"[MeSH Terms]) OR pecan*) OR "pinus"[MeSH Terms]) OR pine nut*) OR "pistacia"[MeSH Terms]) OR pistachio*) OR "arachis"[MeSH Terms]) OR peanut*))

AND

((((((((((((((((((("inflammation"[MeSH Terms]) OR inflammat*) OR endothelial*) OR "adiponectin"[MeSH Terms]) OR adiponectin) OR high molecular weight adiponectin) OR "c reactive protein"[MeSH Terms]) OR c reactive protein) OR c-reactive protein) OR CRP) OR "tumor necrosis factor alpha"[MeSH Terms]) OR tumor necrosis factor*) OR tumour necrosis factor*) OR TNF*) OR "interleukins"[MeSH Terms]) OR interleukin*) OR "cell adhesion molecules"[MeSH Terms]) OR adhesion molecule*) OR flow mediated dilat*) OR flow-mediated dilat*) OR FMD) OR "cytokines"[MeSH Terms]) OR cytokine*)

Supplementary material 3: Differences in CRP, adiponectin, TNF-α, IL-6, ICAM-1, VCAM-1, and FMD following nut consumption, compared to control, using correlation coefficient of 0.5

Outcome	Number of analyses	Number of participants	Effect estimate		Inconsistency (I^2)
CRP (mg/L)	26	1578	-0.03 mg/L [-0.09, 0.03], P = 0.30	-5.53 mg/L [-11.96, 0.90] - 0.60 mg/L [-2.44, 3.64]	33%
Total adiponectin (µg/mL)	7	506	0.15 µg/mL [-0.77, 1.07], P = 0.75	-9.80µg/mL [-23.99, 4.39] - 10.60µg/mL [6.39, 14.81]	81%
TNF-α (pg/mL)	8	482	-0.05 pg/mL [-0.12, 0.02], P = 0.17	-3.70pg/mL [-6.93, -0.47] - 0.70 pg/mL [-0.41, 1.81]	7%
IL-6 (pg/mL)	13	906	-0.06 pg/mL [-0.16, 0.04], P = 0.24	-1.55 pg/mL [-2.80, -0.30] - 0.46 pg/mL [-0.22, 1.14]	28%
ICAM-1 (ng/mL)	15	1047	0.62 ng/mL [-0.24, 1.49], P = 0.16	-80.63ng/mL [-209.62, 48.36] - 16.76ng/mL [1.44, 32.08]	0%
VCAM-1 (ng/mL)	14	804	1.25 ng/mL [-12.09, 14.59], P = 0.85	-99.72ng/mL [-216.35, 116.91] - 62.00ng/mL [-39.40, 163.40]	9%

FMD (%)	9	652	0.74 % [0.27, 1.20], P = 0.002	-0.40% [-1.33, 0.53] - 2.36% [-1.71, 6.43]	46%

Supplementary material 4: Results of sub-group analyses

Table 1: Results of sub-group analyses for CRP

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	17	847	-0.00 mg/L [-0.04, 0.03]	Chi² = 1.02, df = 1 (P = 0.31), I² = 1.9%
	More than three months	9	731	-0.24 mg/L [-0.69, 0.22]	
Risk of bias	Low/unclear	11	588	-0.25 mg/L [-0.53, 0.04]	Chi² = 2.82, df = 1 (P = 0.09), I² = 64.6%
	High	15	990	0.00 mg/L [-0.00, 0.00]	
Nut type	Almond	7	295	-0.79 mg/L [-1.52, -0.06]	Chi² = 10.42, df = 6 (P = 0.11), I² = 42.4%
	Walnut	5	336	0.00 mg/L [-0.00, 0.00]	
	Hazelnut	2	163	-0.31 mg/L [-0.79, 0.18]	
	Mixed nut	5	318	0.01 mg/L [-0.03, 0.05]	
	Peanut	2	187	-0.38 mg/L [-0.89, 0.13]	

	Pistachio	4	258	-0.42 mg/L [-1.03, 0.19]	
	Brazil nut	1	21	-0.15 mg/L [-0.90, 0.60]	
Health status	Healthy	2	61	0.00 mg/L [-0.00, 0.00]	Chi² = 10.41, df = 5 (P = 0.06), I² = 52.0%
	Chronic disease risk factors	14	869	-0.29 mg/L [-0.54, -0.04]	
	T2DM	4	208	-1.18 mg/L [-2.70, 0.35]	
	MetS	4	242	-0.19 mg/L [-0.55, 0.17]	
	CAD	1	90	-0.60 mg/L [-2.53, 1.33]	
	Combination	1	108	0.50 mg/L [-0.34, 1.34]	
Energy value of nuts included in diet	Adjusted	16	1029	-0.23 mg/L [-0.44, -0.01]	Chi² = 3.99, df = 1 (P = 0.05), I² = 74.9%
	Not adjusted	10	549	-0.00 mg/L [-0.06, 0.05]	
Study design	Parallel	14	828	-0.29 mg/L [-0.58, 0.00]	Chi² = 3.84, df = 1 (P = 0.05), I² = 74.0%
	Cross-over	12	750	0.00 mg/L [-0.00, 0.00]	
Nut dose	<50g/day	13	828	0.00 mg/L [-0.00, 0.00]	Chi² = 5.74, df = 1 (P = 0.02), I² = 82.6%
	≥50g/day	13	750	-0.34 mg/L [-0.63, -0.06]	

Table 2: Results of sub-group analyses for FMD

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	6	386	0.77 % [0.17,1.38]	Chi² = 0.01, df = 1 (P = 0.91), I² = 0%
	More than three months	3	266	0.70 % [-0.29, 1.70]	
Risk of bias	Low/unclear	6	480	0.69 % [0.22, 1.16]	Chi² = 1.32, df = 1 (P = 0.25), I² = 24.2%
	High	3	172	1.43 % [0.25, 2.61]	
Nut type	Almond	1	90	0.80 % [-0.75, 2.35]	Chi² = 3.86, df = 2 (P = 0.15), I² = 48.1%
	Walnut	5	404	1.02 % [0.51, 1.53]	
	Pistachio	3	158	-0.11 % [-1.11, 0.90]	
Health status	Chronic disease risk factors	4	230	1.09 % [0.25, 1.92]	Chi² = 0.97, df = 3 (P = 0.81), I² = 0%
	T2DM	2	108	0.38 % [-0.98, 1.74]	

	CAD	1	90	0.80 % [-0.75, 2.35]	
	Combination	2	224	0.60 % [-0.43, 1.62]	
Energy value of nuts included in diet	Adjusted	8	540	0.77 % [0.27, 1.27]	Chi^2 = 0.00, df = 1 (P = 1.00), I^2 = 0%
	Not adjusted	1	112	0.77 % [-0.64, 2.18]	
Study design	Parallel	1	42	2.36 % [-1.71, 6.43]	Chi^2 = 0.58, df = 1 (P = 0.45), I^2 = 0%
	Cross-over	8	610	0.77 % [0.32, 1.21]	
Nut dose	<50g/day	1	42	2.36 % [-1.71, 6.43]	Chi^2 = 0.58, df = 1 (P = 0.45), I^2 = 0%
	≥50g/day	8	610	0.77 % [0.32, 1.21]	

Table 3: Results of sub-group analyses for adiponectin

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	2	130	-0.60 µg/mL [-2.48, 1.28]	Chi² = 1.03, df = 1 (P = 0.31), I² = 3.3%
	More than three months	5	376	1.71 µg/mL [-2.33, 5.75]	
Risk of bias	Low/unclear	3	234	-0.00 µg/mL [-0.00, 0.00]	Chi² = 0.45, df = 1 (P = 0.50), I² = 0%
	High	4	272	1.91 µg/mL [-3.70, 7.53]	
Nut type	Walnut	2	96	-0.52 µg/mL [-3.78, 2.75]	Chi² = 0.57, df = 2 (P = 0.75), I² = 0%
	Mixed nut	3	234	-0.00 µg/mL [-0.00, 0.00]	
	Pistachio	2	176	4.49 µg/mL [-8.30, 17.28]	
Health status	Chronic disease risk factors	2	178	-2.33 µg/mL [-5.28, 0.63]	Chi² = 3.42, df = 2 (P = 0.18), I² = 41.5%
	MetS	3	178	0.53 µg/mL [-0.49, 1.55]	

	Combination	2	150	-2.05 μg/mL [-11.64, 7.54]	
Energy value of nuts included in diet	Adjusted	5	396	0.80 μg/mL [-4.62, 6.22]	Chi² = 0.08, df = 1 (P = 0.77), I² = 0%
	Not adjusted	2	110	-0.00 μg/mL [-0.00, 0.00]	
Study design	Parallel	5	328	0.53 μg/mL [-0.43, 1.49]	Chi² = 3.24, df = 1 (P = 0.07), I² = 69.2%
	Cross-over	2	178	-2.33 μg/mL [-5.28, 0.63]	
Nut dose	<50g/day	6	398	0.34 μg/mL [-0.60, 1.28]	Chi² = 0.49, df = 1 (P = 0.48), I² = 0%
	≥50g/day	1	108	-2.48 μg/mL [-10.31, 5.35]	

Table 4: Results of sub-group analyses for TNF-α

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	5	285	-0.06 pg/mL [-0.12, 0.01]	Chi² = 0.21, df = 1 (P = 0.65), I² = 0%
	More than three months	3	197	-0.70 pg/mL [-3.48, 2.08]	
Risk of bias	Low/unclear	2	148	0.11 pg/mL [-0.51, 0.73]	Chi² = 0.21, df = 1 (P = 0.65), I² = 0%
	High	6	334	-0.04 pg/mL [-0.22, 0.15]	
Nut type	Almond	3	151	-0.06 pg/mL [-0.13, 0.01]	Chi² = 6.75, df = 4 (P = 0.15), I² = 40.8%
	Walnut	2	90	-0.03 pg/mL [-0.21, 0.14]	
	Mixed nut	1	108	0.70 pg/mL [-0.41, 1.81]	
	Peanut	1	65	-0.16 pg/mL [-1.41, 1.10]	
	Pistachio	1	68	-3.70 pg/mL [-6.93, -0.47]	
Health status	Healthy	1	40	-0.01 pg/mL [-0.24, 0.22]	Chi² = 7.08, df = 5 (P = 0.21), I² =

	Chronic disease risk factors	2	115	-0.07 pg/mL [-0.34, 0.20]	29.4%
	T2DM	2	61	-0.06 pg/mL [-0.13, 0.01]	
	MetS	1	68	-3.70 pg/mL [-6.93, -0.47]	
	CAD	1	90	0.10 pg/mL [-0.54, 0.74]	
	Combination	1	108	0.70 pg/mL [-0.41, 1.81]	
Energy value of nuts included in diet	Adjusted	6	421	-0.04 pg/mL [-0.24, 0.15]	Chi^2 = 0.05, df = 1 (P = 0.83), I^2 = 0%
	Not adjusted	2	61	-0.01 pg/mL [-0.24, 0.22]	
Study design	Parallel	4	262	-0.27 pg/mL [-1.68, 1.14]	Chi^2 = 0.09, df = 1 (P = 0.77), I^2 = 0%
	Cross-over	4	220	-0.05 pg/mL [-0.12, 0.01]	
Nut dose	<50g/day	5	287	-0.02 pg/mL [-0.34, 0.31]	Chi^2 = 0.06, df = 1 (P = 0.80), I^2 = 0%
	≥50g/day	3	195	-0.06 pg/mL [-0.13, 0.01]	

Table 5: Results of sub-group analyses for IL-6

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	7	386	0.04 pg/mL [-0.02, 0.09]	Chi² = 2.71, df = 1 (P = 0.10), I² = 63.1%
	More than three months	6	520	-0.19 pg/mL [-0.45, 0.07]	
Risk of bias	Low/unclear	5	314	-0.01 pg/mL [-0.26, 0.23]	Chi² = 0.62, df = 1 (P = 0.43), I² = 0%
	High	8	592	-0.13 pg/mL [-0.29, 0.03]	
Nut type	Almond	4	201	-0.16 pg/mL [-0.44, 0.13]	Chi² = 5.17, df = 4 (P = 0.27), I² = 22.6%
	Walnut	3	216	-0.11 pg/mL [-0.31, 0.10]	
	Hazelnut	2	163	0.05 pg/mL [-0.01, 0.11]	
	Mixed nut	3	218	-0.18 pg/mL [-0.99, 0.63]	
	Pistachio	1	108	-0.14 pg/mL [-0.47, 0.19]	
Health status	Chronic disease risk	6	497	0.04 pg/mL [-0.02, 0.10]	Chi² = 3.09, df = 5 (P = 0.69), I² = 0%

	factors				
	Healthy	1	40	-0.10 pg/mL [-0.39, 0.19]	
	MetS	2	110	-0.47 pg/mL [-2.44, 1.49]	
	T2DM	2	61	-0.14 pg/mL [-0.46, 0.18]	
	CAD	1	90	-0.50 pg/mL [-1.62, 0.62]	
	Combination	1	108	0.00 pg/mL [-0.41, 0.41]	
Energy value of nuts included in diet	Adjusted	8	628	0.03 pg/mL [-0.02, 0.09]	Chi^2 = 0.68, df = 1 (P = 0.41), I^2 = 0%
	Not adjusted	5	278	-0.18 pg/mL [-0.68, 0.32]	
Study design	Parallel	7	528	-0.04 pg/mL [-0.29, 0.22]	Chi^2 = 0.26, df = 1 (P = 0.61), I^2 = 0%
	Cross-over	6	378	-0.12 pg/mL [-0.27, 0.04]	
Nut dose	<50g/day	9	618	-0.03 pg/mL [-0.17, 0.12]	Chi^2 = 0.65, df = 1 (P = 0.42), I^2 = 0%
	≥50g/day	4	288	-0.14 pg/mL [-0.36, 0.09]	

Table 6: Results of sub-group analyses for ICAM-1

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	12	537	0.66 ng/mL [-0.56, 1.88]	$Chi^2 = 0.04$, df = 1 (P = 0.83), I^2 = 0%
	More than three months	3	510	2.35 ng/mL [-13.26, 17.96]	
Risk of bias	Low/unclear	8	660	4.58 ng/mL [-2.68, 11.85]	$Chi^2 = 1.14$, df = 1 (P = 0.29), I^2 = 12.4%
	High	7	387	0.57 ng/mL [-0.66, 1.80]	
Nut type	Almond	3	81	11.65 ng/mL [-1.49, 24.80]	$Chi^2 = 3.34$, df = 4 (P = 0.50), I^2 = 0%
	Walnut	5	244	0.58 ng/mL [-0.65, 1.81]	
	Hazelnut	2	163	-3.32 ng/mL [-22.42, 15.78]	
	Mixed nut	4	499	3.75 ng/mL [-7.31, 14.81]	
	Pistachio	1	60	-2.60 ng/mL [-18.13, 12.93]	

Health status	Healthy	1	40	0.65 ng/mL [-0.59, 1.89]	Chi² = 1.02, df = 4 (P = 0.91), I² = 0%
	Chronic disease risk factors	9	444	0.86 ng/mL [-6.94, 8.65]	
	T2DM	2	100	-1.67 ng/mL [-16.50, 13.16]	
	MetS	2	110	-13.46 ng/mL [-76.61, 49.70]	
	Combination	1	353	8.00 ng/mL [-8.85, 24.85]	
Energy value of nuts included in diet	Adjusted	9	749	-1.31 ng/mL [-8.90, 6.29]	Chi² = 0.48, df = 1 (P = 0.49), I² = 0%
	Not adjusted	6	298	2.06 ng/mL [-3.72, 7.84]	
Study design	Parallel	7	667	5.39 ng/mL [-2.46, 13.24]	Test for subgroup differences: Chi² = 1.42, df = 1 (P = 0.23), I² = 29.6%
	Cross-over	8	380	0.56 ng/mL [-0.66, 1.79]	
Nut dose	<50g/day	9	830	0.62 ng/mL [-0.60, 1.84]	Chi² = 0.29, df = 1 (P = 0.59), I² = 0%
	≥50g/day	6	217	3.66 ng/mL [-7.32, 14.65]	

Table 7: Results of sub-group analyses for VCAM-1

Sub-group analysis category	Sub-group	Number of analyses	Number of participants	Effect estimate	Test for sub-group differences
Duration	Less than three months	11	537	2.23 ng/mL [-9.68, 14.13]	$Chi^2 = 0.02$, df = 1 (P = 0.89), I^2 = 0%
	More than three months	3	267	-4.16 ng/mL [-96.76, 88.44]	
Risk of bias	Low/unclear	8	417	2.39 ng/mL [-9.72, 14.50]	$Chi^2 = 0.04$, df = 1 (P = 0.83), I^2 = 0%
	High	6	387	7.42 ng/mL [-38.20, 53.04]	
Nut type	Almond	4	171	1.11 ng/mL [-13.10, 15.33]	$Chi^2 = 1.56$, df = 4 (P = 0.82), I^2 = 0%
	Walnut	3	154	-30.19 ng/mL [-99.92, 39.53]	
	Hazelnut	2	163	17.62 ng/mL [-24.61, 59.85]	
	Mixed nut	4	256	9.30 ng/mL [-21.20, 39.80]	
	Pistachio	1	60	3.40 ng/mL [-60.84, 67.64]	

Health status	Chronic disease risk factors	8	394	3.95 ng/mL [-9.12, 17.02]	Chi² = 2.08, df = 4 (P = 0.72), I² = 0%
	T2DM	2	100	-17.58 ng/mL [-67.98, 32.82]	
	MetS	2	110	9.61 ng/mL [-23.37, 42.59]	
	CAD	1	90	-48.00 ng/mL [-193.52, 97.52]	
	Combination	1	110	-70.00 ng/mL [-230.43, 90.43]	
Energy value of nuts included in diet	Adjusted	9	546	-12.78 ng/mL [-42.38, 16.83]	Chi² = 1.27, df = 1 (P = 0.26), I² = 21.0%
	Not adjusted	5	258	5.71 ng/mL [-7.00, 18.42]	
Study design	Parallel	7	424	5.01 ng/mL [-7.27, 17.29]	Chi² = 1.26, df = 1 (P = 0.26), I² = 20.5%
	Cross-over	7	380	-17.66 ng/mL [-55.33, 20.02]	
Nut dose	<50g/day	7	497	9.74 ng/mL [-14.01, 33.49]	Chi² = 0.43, df = 1 (P = 0.51), I² = 0%
	≥50g/day	7	307	0.63 ng/mL [-12.78, 14.04]	

Supplementary material 5: Forest plots of difference in CRP after exclusion of individual studies

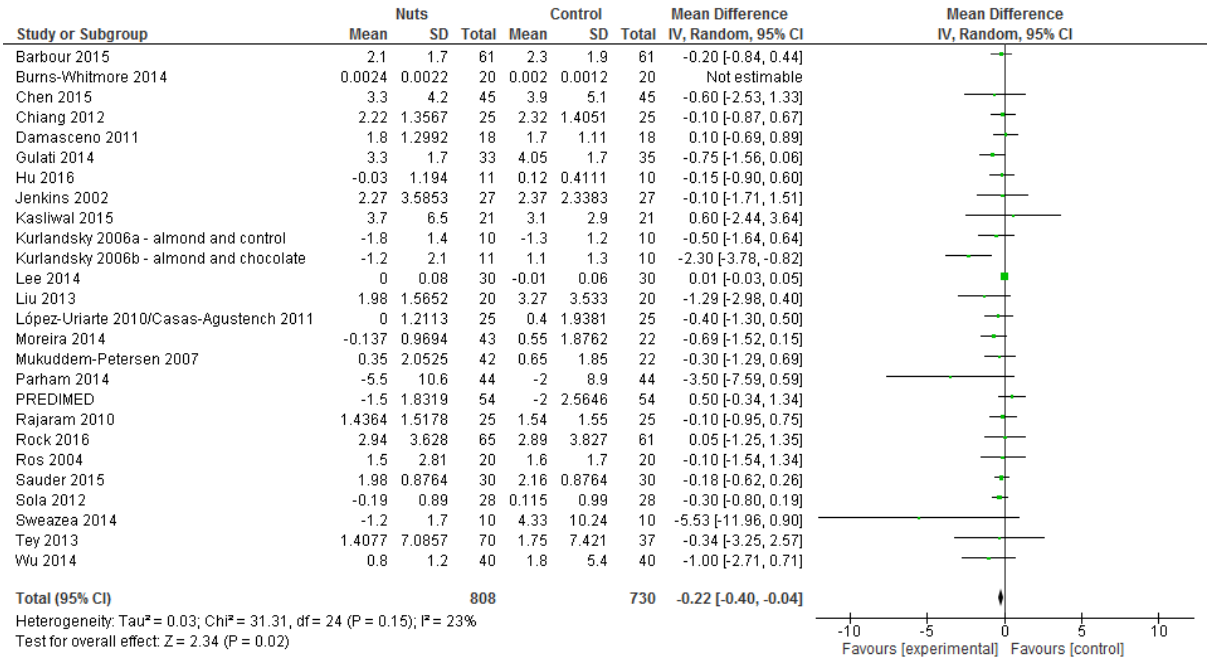

Figure 1: Difference in CRP (mg/L) between nut consumption and control, after exclusion of Burns-Whitmore et al. (2014). Diamond indicates weighted mean difference with 95% confidence intervals.

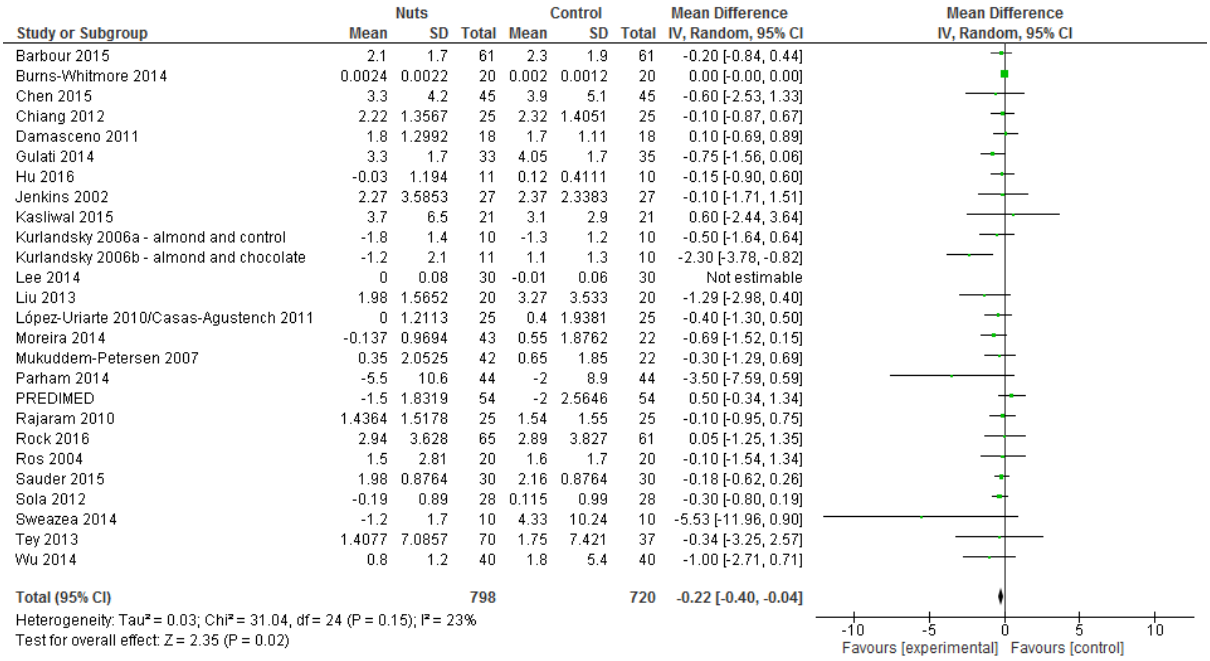

Figure 2: Difference in CRP (mg/L) between nut consumption and control, after exclusion of Lee et al. (2014). Diamond indicates weighted mean difference with 95% confidence intervals.

Supplementary material 6: Forest plots of differences in biomarkers between nut consumption and control

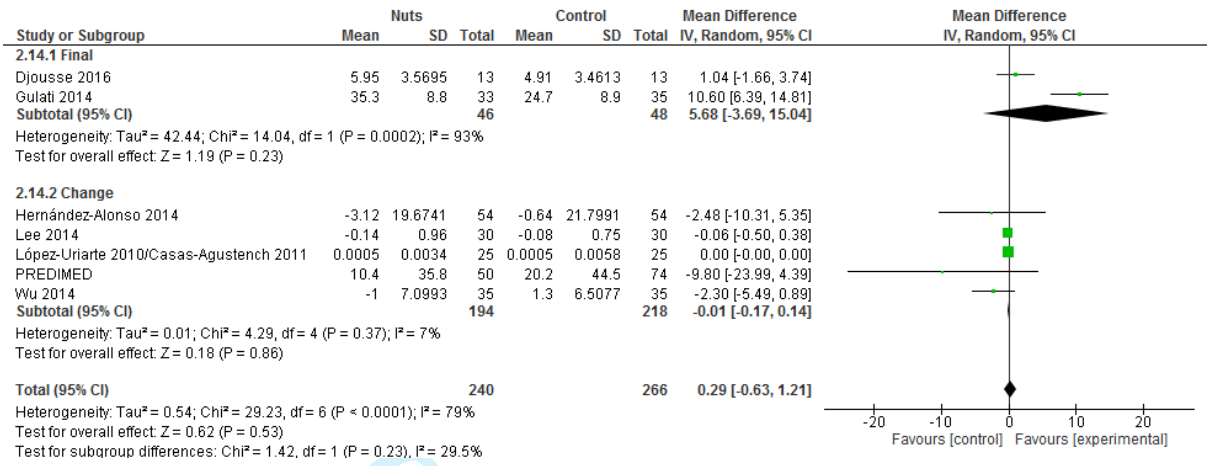

Figure 3: Difference in adiponectin (µg/mL) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals.

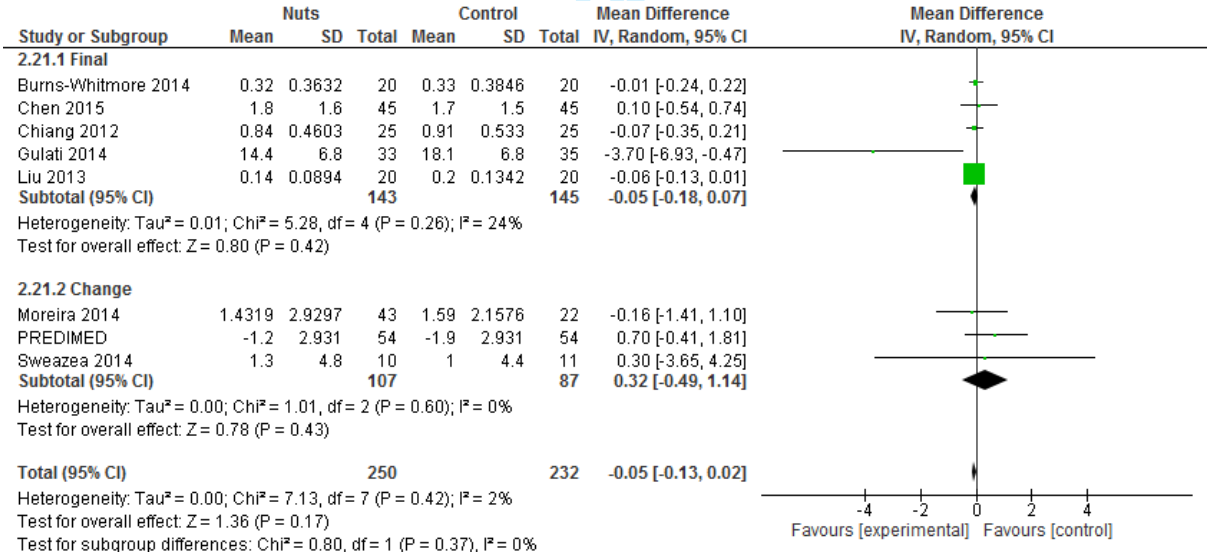

Figure 4: Difference in TNF-α (pg/mL) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals.

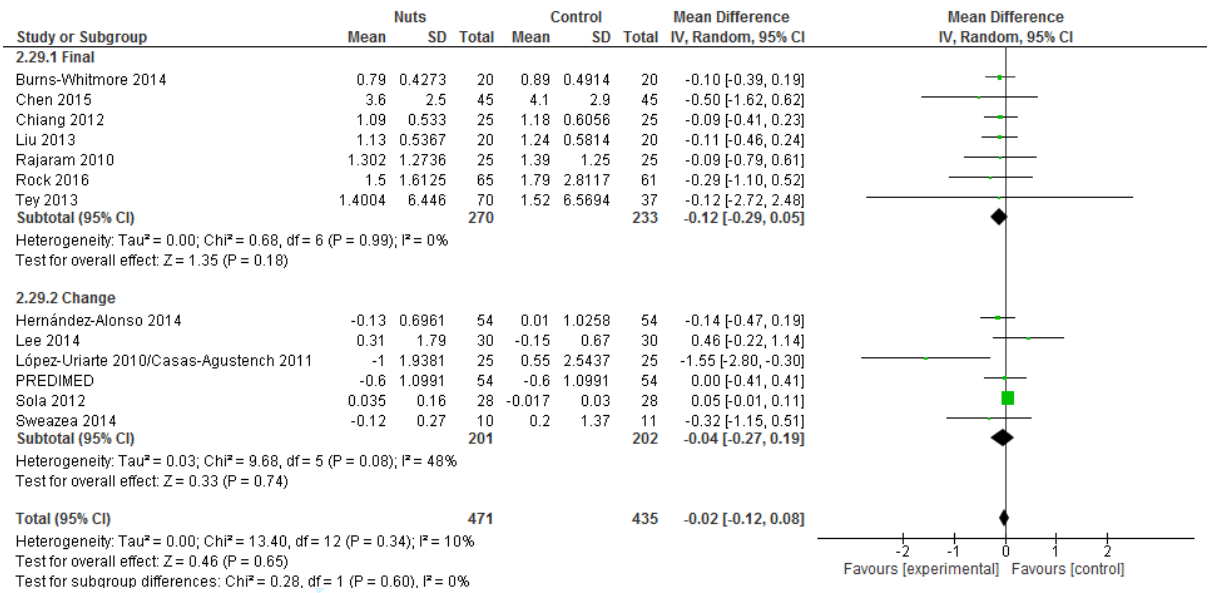

Figure 5: Difference in IL-6 (pg/mL) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals

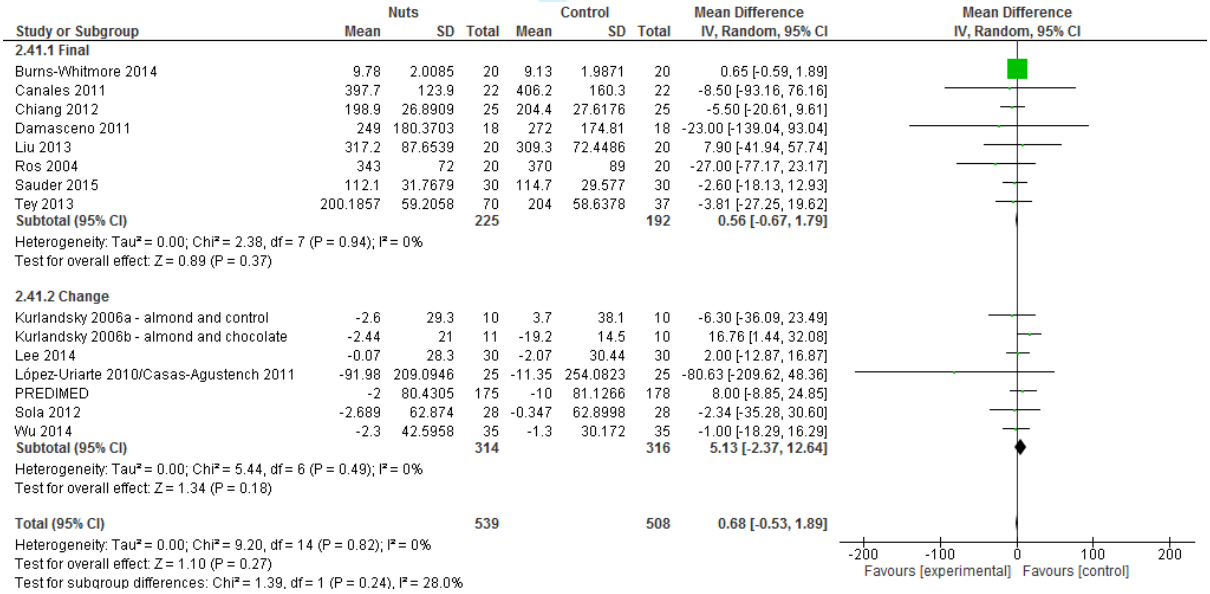

Figure 6: Difference in ICAM-1 (ng/mL) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals

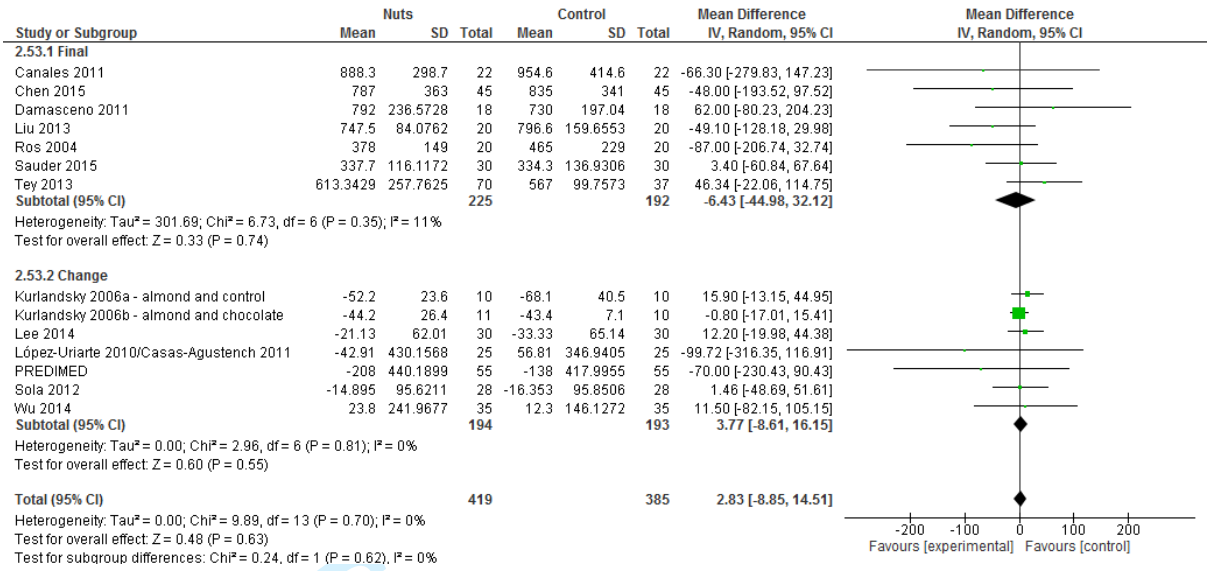

Figure 7: Difference in VCAM-1 (ng/mL) between nut consumption and control (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals

Supplementary material 7: Funnel plots

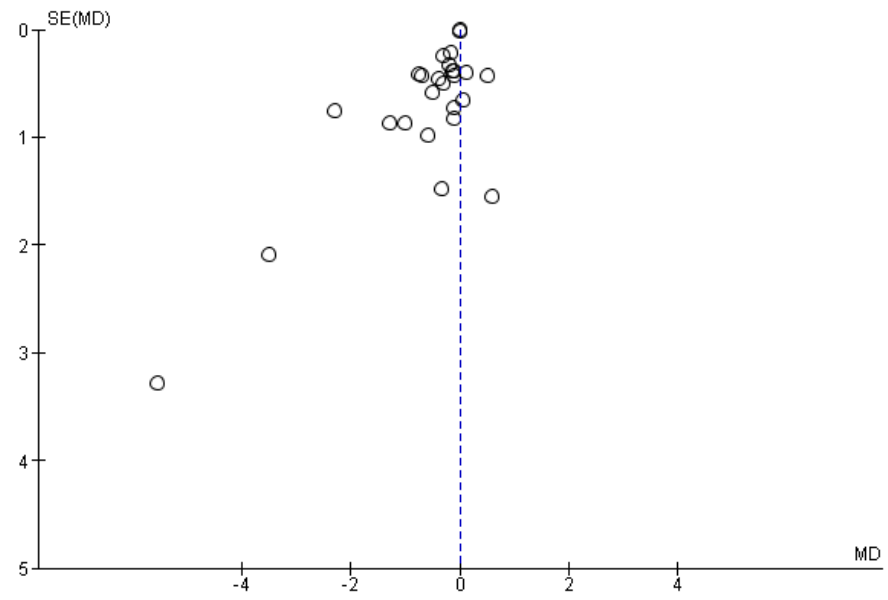

Figure 8: Funnel plot of the effect of nut consumption on CRP (mg/L)

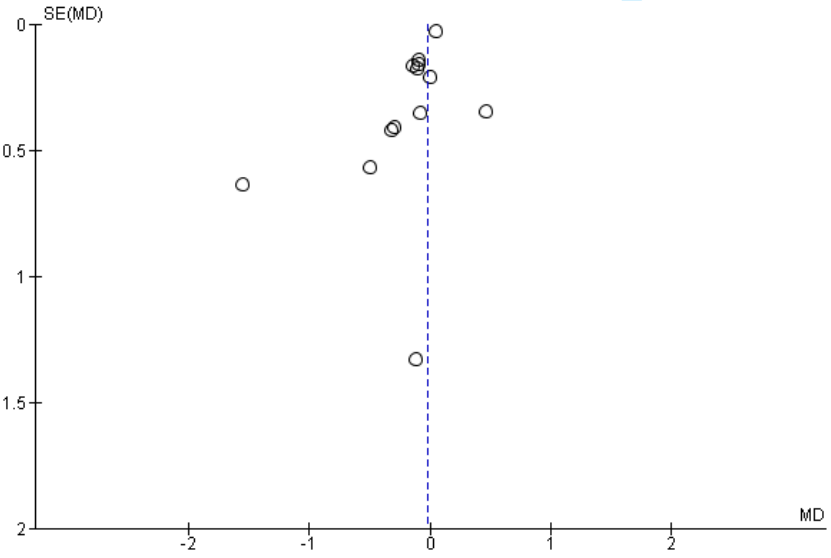

Figure 9: Funnel plot of the effect of nut consumption on IL-6 (pg/mL)

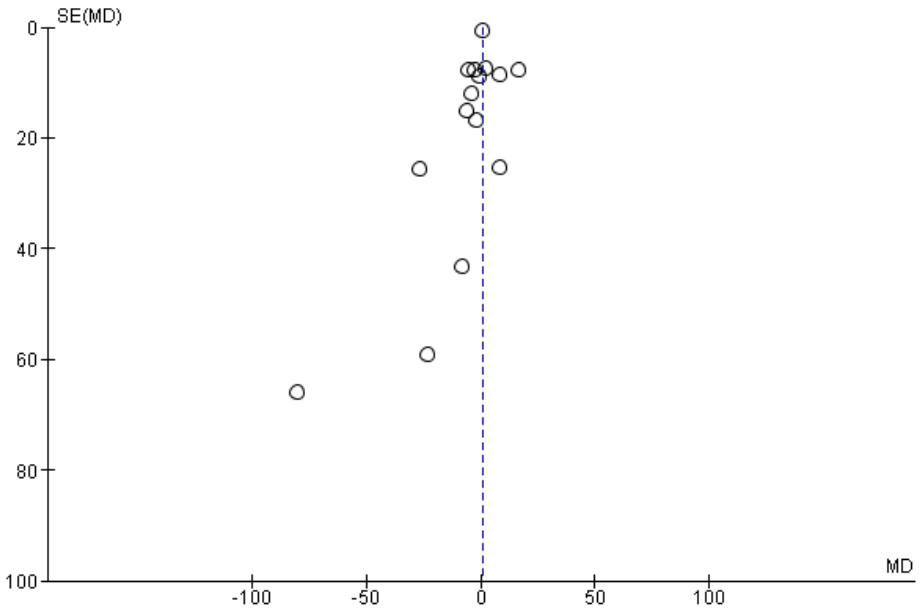

Figure 10: Funnel plot of the effect of nut consumption on ICAM-1 (ng/mL)

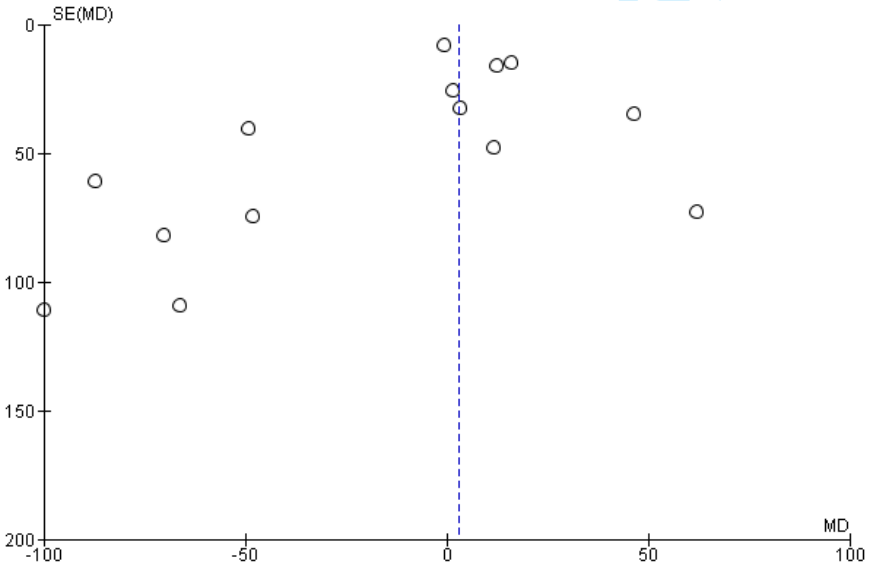

Figure 11: Funnel plot of the effect of nut consumption on VCAM-1 (ng/mL)

Supplementary material 8: Risk of bias assessment summary

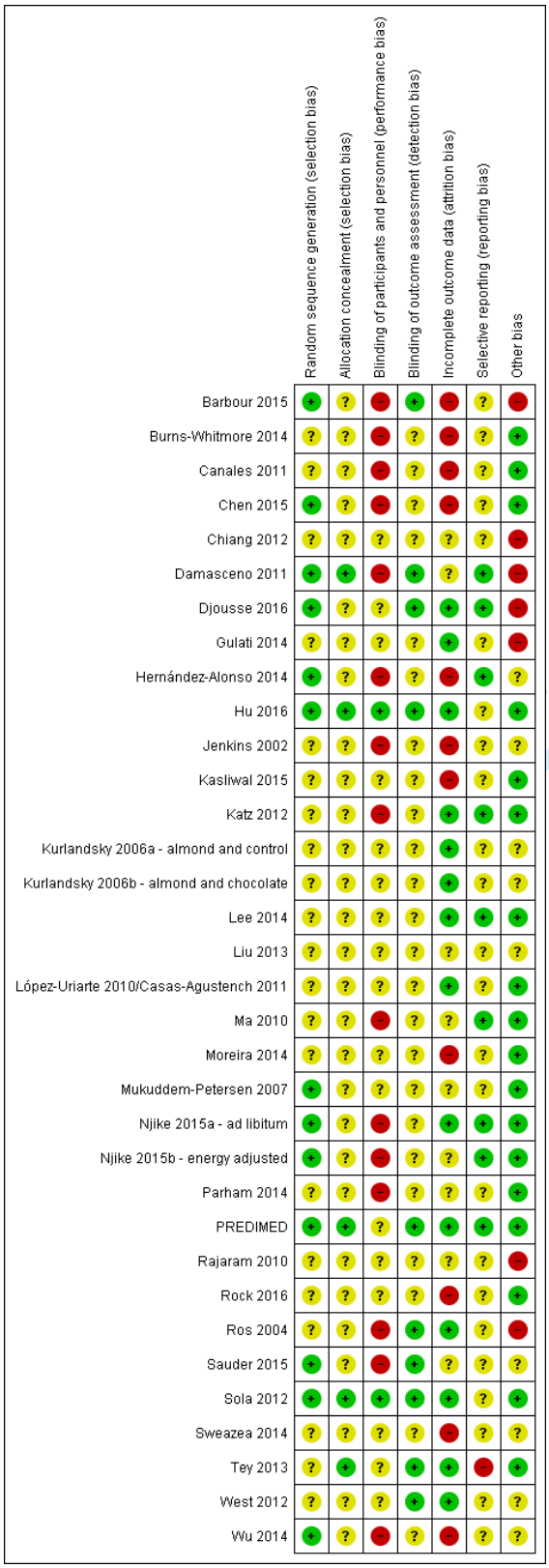

Figure 12: Risk of bias assessment for each study

Supplementary material 9: Justification for risk of bias judgements

Barbour et al., 2015

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Article states: "Subjects were randomised using computer generated software"
Allocation concealment (selection bias)	Unclear risk	Not specified
Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Low risk	Article states: "Data entry and analysis was blinded to minimise investigator bias"
Incomplete outcome data (attrition bias)	High risk	>10% withdrawal, intention-to-treat (ITT) not used
Selective reporting (reporting bias)	Unclear risk	ANZCTRN registration available, includes pre-specified outcomes not reported in this paper but which may have been reported in unpublished primary paper
Other bias	High risk	No washout period - authors specify 12 week period would have been sufficient to avoid carry over effects but this is not clear

Burns-Whitmore et al., 2014

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, method not given
Allocation concealment (selection bias)	Unclear risk	Not specified

Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although outcomes unlikely to be influenced by blinding
Incomplete outcome data (attrition bias)	High risk	>20% withdrawal, ITT not used (not clear which group participants dropped out of)
Selective reporting (reporting bias)	Unclear risk	Protocol not available
Other bias	Low risk	4 week wash-out period (justified). Did not report baseline results for outcomes of interest, but unlikely to influence as cross-over study

Canales et al., 2011

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, method not given
Allocation concealment (selection bias)	Unclear risk	Not specified
Blinding of participants and personnel (performance bias)	High risk	Stated to be non-blinded. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although outcomes unlikely to be influenced by blinding
Incomplete outcome data (attrition bias)	High risk	>10% withdrawal, ITT not used
Selective reporting (reporting bias)	Unclear risk	Protocol not available
Other bias	Low risk	4 -6 week wash-out period (appears suitable)

Chen et al., 2015

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	The program in the randomization.com was employed for the randomization
Allocation concealment (selection bias)	Unclear risk	Not specified
Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although outcomes unlikely to be influenced by blinding
Incomplete outcome data (attrition bias)	High risk	>10% withdrawal, ITT not used
Selective reporting (reporting bias)	Unclear risk	Clinical trial registration provides insufficient detail to determine if all outcomes reported
Other bias	Low risk	Wash-out period of 4 weeks appears suitable

Chiang et al., 2012

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, method not given
Allocation concealment (selection bias)	Unclear risk	Not specified
Blinding of participants and personnel (performance bias)	Unclear risk	single-blinded, unclear who was blinded (participants vs personnel) as all foods provided
Blinding of outcome assessment (detection bias)	Unclear risk	Stated to be single-blind (assume outcome assessors), outcomes unlikely to be influenced by blinding
Incomplete outcome data (attrition bias)	Unclear risk	<10%, however unclear at which point withdrew
Selective reporting (reporting bias)	Unclear risk	Protocol not available

Other bias	High risk	Wash-out period of 2 days

Damasceno et al., 2011

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomization was simple (not stratified) and was based on a random number table prepared by a biostatistician
Allocation concealment (selection bias)	Low risk	"...six possible diet sequences, which were coded and introduced into sealed envelopes"
Blinding of participants and personnel (performance bias)	High risk	Stated as not possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Low risk	Investigators involved in preparation of databases and laboratory determinations, however, were masked with respect to treatment sequence
Incomplete outcome data (attrition bias)	Unclear risk	<10%, however unclear at which point withdrew
Selective reporting (reporting bias)	Low risk	The study protocol is available and all pre-specified outcomes of interest to the review have been reported in the pre-specified way
Other bias	High risk	No washout period. Authors state would not effect, but likely to be carry-over effect

Djousse et al., 2016

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Article states: "computer-generated randomization schedule with balanced blocks, stratified by prevalent DM and coronary artery disease"

Allocation concealment (selection bias)	Unclear risk	Biostatistician generated schedule and did not have contact with study subjects, but not clear how allocation was communicated to researchers
Blinding of participants and personnel (performance bias)	Unclear risk	Unclear if participants blinded, researcher providing intervention not blinded
Blinding of outcome assessment (detection bias)	Low risk	Test completed by blinded staff
Incomplete outcome data (attrition bias)	Low risk	<5% withdrawal
Selective reporting (reporting bias)	Low risk	The study protocol is available and all pre-specified outcomes of interest to the review have been reported in the pre-specified way
Other bias	High risk	Control group had significantly higher proportion with hypercholesterolaemia

Gulati et al., 2014

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, however no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	No details given
Blinding of participants and personnel (performance bias)	Unclear risk	Not stated if participants blinded, would not be possible to blind personnel
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	Low risk	12% drop-out, but similar between groups and ITT used
Selective reporting (reporting bias)	Unclear risk	protocol not available
Other bias	High risk	CRP significantly higher in control group at baseline

Hernández-Alonso et al., 2014

Bias	**Authors' judgement**	**Support for judgement**

Random sequence generation (selection bias)	Low risk	Article states: "randomly assigned to one of the two different intervention periods using a computer generated random number table"
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, however would be unlikely to affect results
Incomplete outcome data (attrition bias)	High risk	10% drop-out (ITT used) - but all dropped out during first pistachio sequence
Selective reporting (reporting bias)	Low risk	The study protocol is available and all pre-specified outcomes of interest to the review have been reported in the pre-specified way
Other bias	Unclear risk	2 week washout period, unclear if sufficient

Hu et al., 2016

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Randomisation sequence was computer generated
Allocation concealment (selection bias)	Low risk	Study states: "Allocation concealment was achieved by keeping codes in a sealed envelope by a person who was not in contact with study subjects, and codes were disclosed after the study"
Blinding of participants and personnel (performance bias)	Low risk	Study states: "It was impossible to blind participants because of the nature of the intervention (especially the Brazil nuts), but all data curation, checking, measurements and data analysis were conducted by researchers blinded to treatment allocation of subjects."

Blinding of outcome assessment (detection bias)	Low risk	Study states: "It was impossible to blind participants because of the nature of the intervention (especially the Brazil nuts), but all data curation, checking, measurements and data analysis were conducted by researchers blinded to treatment allocation of subjects."
Incomplete outcome data (attrition bias)	Low risk	<10% drop-out and evenly spread between groups
Selective reporting (reporting bias)	Unclear risk	Protocol available, but not possible to determine if all outcomes reported
Other bias	Low risk	

Jenkins et al., 2002

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, however would be unlikely to affect results
Incomplete outcome data (attrition bias)	High risk	>20% drop-out, and unclear at which point in study participants dropped out
Selective reporting (reporting bias)	High risk	Study protocol is available but unclear if all relevant outcomes have not been reported
Other bias	Unclear risk	2 week washout period, unclear if sufficient

Kasliwal et al., 2015

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	Unclear risk	"open-label", unclear if both participants and personnel unblinded
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	High risk	>20% drop-out rate, ITT not used
Selective reporting (reporting bias)	Unclear risk	protocol not available
Other bias	Low risk	

Katz et al., 2012

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Unclear risk	Single-blinded (unclear who was blinded though), although would be unlikely to affect results
Incomplete outcome data (attrition bias)	Low risk	13% dropout (ITT used), but similar between groups
Selective reporting (reporting bias)	Low risk	The study protocol is available and all pre-specified outcomes of interest to the review have been reported in the pre-specified way

Other bias	Low risk	Wash-out period of 4 weeks appears suitable

Kurlandsky 2006a - almond and control

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	Unclear risk	Not possible to blind personnel, unclear if participants blinded
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	Low risk	<5% dropout, although not clear which group dropped out of
Selective reporting (reporting bias)	Unclear risk	protocol not available
Other bias	Unclear risk	Age differed significantly between groups, unclear if impacted on results

Kurlandsky 2006b - almond and chocolate

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	Unclear risk	Not possible to blind personnel, unclear if participants blinded
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	Low risk	<5% dropout, although not clear which group dropped out of
Selective reporting (reporting bias)	Unclear risk	protocol not available

Other bias	Unclear risk	Age differed significantly between groups, unclear if impacted on results

Lee et al., 2014

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	Unclear risk	Not possible to blind personnel, unclear if participants blinded
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	Low risk	<5% dropout, group specified
Selective reporting (reporting bias)	Low risk	The study protocol is available and all pre-specified outcomes of interest to the review have been reported in the pre-specified way
Other bias	Low risk	No differences in baseline characteristics

Liu et al., 2013

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	Unclear risk	Unclear if blinded as all foods provided
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	Unclear risk	<10% dropout, but unclear during which diet participant dropped out

Selective reporting (reporting bias)	Unclear risk	protocol not available
Other bias	Unclear risk	2 week washout period, unclear if sufficient

López-Uriarte et al., 2010/Casas-Agustench et al., 2011

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, method not given
Allocation concealment (selection bias)	Unclear risk	Not specified
Blinding of participants and personnel (performance bias)	Unclear risk	Not possible to blind personnel, unclear if participants blinded
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although outcomes unlikely to be influenced by blinding
Incomplete outcome data (attrition bias)	Low risk	<5% withdrawal
Selective reporting (reporting bias)	Unclear risk	Clinical trial registration provides insufficient detail to determine if all outcomes reported
Other bias	Low risk	

Ma et al., 2010

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods

Blinding of outcome assessment (detection bias)	Unclear risk	Single-blinded (unclear if all outcome assessors blinded), although would be unlikely to affect results
Incomplete outcome data (attrition bias)	Unclear risk	<10% dropout, ITT used (although unclear when participants dropped out)
Selective reporting (reporting bias)	Low risk	The study protocol is available and all pre-specified outcomes of interest to the review have been reported in the pre-specified way
Other bias	Low risk	8 week washout appears adequate

Moreira et al., 2014

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	Unclear risk	Not possible to blind personnel, unclear if participants blinded
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	High risk	>10% drop out/excluded, not evenly spread across groups
Selective reporting (reporting bias)	Unclear risk	protocol not available
Other bias	Low risk	

Mukuddem-Petersen et al., 2007

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Drawing numbers from a hat
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	Unclear risk	Not possible to blind personnel, unclear if participants blinded

Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	Unclear risk	<10% drop-out, but unclear during which diet participants dropped out
Selective reporting (reporting bias)	Unclear risk	protocol not available
Other bias	Low risk	

Njike et al., 2015a – non-calorie adjusted

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	study participants were randomized using a SAS-generated random table
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, however would be unlikely to affect results
Incomplete outcome data (attrition bias)	Low risk	>10% drop-out, but ITT and similar between groups
Selective reporting (reporting bias)	Low risk	The study protocol is available and all pre-specified outcomes of interest to the review have been reported in the pre-specified way
Other bias	Low risk	

Njike et al., 2015b – calorie adjusted

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	study participants were randomized using a SAS-generated random table

Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, however would be unlikely to affect results
Incomplete outcome data (attrition bias)	Unclear risk	14% drop-out (ITT used) but 3 x in walnut arm
Selective reporting (reporting bias)	Low risk	The study protocol is available and all pre-specified outcomes of interest to the review have been reported in the pre-specified way
Other bias	Low risk	

Parham et al., 2014

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Allocation based on random numbers, but not clear how generated
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	Unclear risk	<10%, but not clear when participants withdrew/were excluded
Selective reporting (reporting bias)	Unclear risk	protocol not available

Other bias	Low risk	washout period of 8 weeks appears appropriate

PREDIMED

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Article states: "Randomization was performed centrally by means of a computer-generated random-number sequence"
Allocation concealment (selection bias)	Low risk	"These tables have been centrally elaborated by the Coordinating Unit and provide a stratified random sequence of allocation for each FC using closed envelopes"
Blinding of participants and personnel (performance bias)	Unclear risk	single-blinded
Blinding of outcome assessment (detection bias)	Low risk	Outcome assessors blinded
Incomplete outcome data (attrition bias)	Low risk	participants completers only
Selective reporting (reporting bias)	Low risk	The study protocol is available and all pre-specified outcomes of interest to the review have been reported in the pre-specified way
Other bias	Low risk	

Rajaram et al., 2010

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	3 x 3 Latin square design, no description of method of randomisation
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	Unclear risk	single-blinded, unclear if participants aware as all foods provided

Blinding of outcome assessment (detection bias)	Unclear risk	single-blind (not stated who blinded), although would be unlikely to affect results
Incomplete outcome data (attrition bias)	Unclear risk	<10%, but not clear when participants withdrew/were excluded
Selective reporting (reporting bias)	Unclear risk	protocol not available
Other bias	High risk	washout period not included, Sabate paper states lipids would stabilise but would still impact starting levels

Rock et al., 2016

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Randomised by study statistician, not clear if involved in other aspects of study
Blinding of participants and personnel (performance bias)	Unclear risk	Not possible to blind personnel, unclear if participants blinded
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	High risk	18% withdrawal, does not appear that ITT used for biomarkers analysis (Table 3)
Selective reporting (reporting bias)	Unclear risk	Protocol is available, but insufficient detail to determine if all outcomes reported
Other bias	Low risk	

Ros et al., 2004

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Randomised but no additional detail given
Allocation concealment (selection bias)	Unclear risk	Not stated

Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention
Blinding of outcome assessment (detection bias)	Low risk	Blinded
Incomplete outcome data (attrition bias)	Low risk	<5% dropout (although not clear when dropped out)
Selective reporting (reporting bias)	Unclear risk	protocol not available
Other bias	High risk	washout period not included, references paper stating lipids would stabilise but would still

Sauder et al., 2015

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Generated via randomization.com
Allocation concealment (selection bias)	Unclear risk	Generated by study coordinator, but not stated if concealed
Blinding of participants and personnel (performance bias)	High risk	"But due to the nature of the dietary intervention, participants were aware of their treatment order assignment"
Blinding of outcome assessment (detection bias)	Low risk	Technicians who measured outcome variables were blinded to treatment assignments
Incomplete outcome data (attrition bias)	Unclear risk	11.7% drop-out, but not clear when participants dropped out
Selective reporting (reporting bias)	Unclear risk	Protocol is available, but insufficient detail to determine if all outcomes reported
Other bias	Unclear risk	washout period of 2 weeks

Sola et al., 2012

Bias	**Authors' judgement**	**Support for judgement**

Random sequence generation (selection bias)	Low risk	The randomization code was computer-generated random number sequence in gender-stratified blocks
Allocation concealment (selection bias)	Low risk	Center and treatment assignment codes were allocated via an interactive electronic response system administered by the Barcelona Randomization Unit, which was not further involved in the study.
Blinding of participants and personnel (performance bias)	Low risk	The participants, clinical investigators and laboratory personnel were blinded with respect to the type of cream being consumed
Blinding of outcome assessment (detection bias)	Low risk	The participants, clinical investigators and laboratory personnel were blinded with respect to the type of cream being consumed
Incomplete outcome data (attrition bias)	Low risk	<10% dropout, similar between groups, ITT used
Selective reporting (reporting bias)	Unclear risk	Protocol is available, but insufficient detail to determine if all outcomes reported
Other bias	Low risk	No differences in baseline characteristics

Sweazea et al., 2014

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	Unclear risk	Not possible to blind personnel, unclear if participants blinded
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	High risk	>10% drop out, ITT not used
Selective reporting (reporting bias)	Unclear risk	protocol not available
Other bias	Unclear risk	Unclear if baseline inflammation levels differ between groups

Tey et al., 2013

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Details of randomisation given, but not how sequence was generated
Allocation concealment (selection bias)	Low risk	Managed by an off-site statistician
Blinding of participants and personnel (performance bias)	Unclear risk	Not possible to blind personnel, unclear if participants blinded
Blinding of outcome assessment (detection bias)	Low risk	Stated to be blinded
Incomplete outcome data (attrition bias)	Low risk	5% drop-out, ITT used, similar drop-out between groups
Selective reporting (reporting bias)	High risk	TNF-α referenced in protocol, not reported in paper.
Other bias	Low risk	controlled for baseline values

West et al., 2012

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated to be randomised, but no further detail given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	Unclear risk	Unclear if blinded as all foods provided
Blinding of outcome assessment (detection bias)	Low risk	Appears to be blinded (Gebauer et al., 2008)
Incomplete outcome data (attrition bias)	Low risk	<5% drop-out (although not clear which group dropped out of)
Selective reporting (reporting bias)	Unclear risk	Protocol not available
Other bias	Unclear risk	2 weeks compliance break (assume washout)

Wu et al., 2014

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	computer generated randomisation sequence

Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and personnel (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated, although would be unlikely to affect results
Incomplete outcome data (attrition bias)	High risk	~20% drop-out
Selective reporting (reporting bias)	Unclear risk	Protocol available, but not possible to determine if all outcomes reported
Other bias	Unclear risk	2 weeks washout

Supplementary material 10: GRADE assessment of the quality of the body of evidence

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	nut consumption	control	Relative (95% CI)	Absolute (95% CI)		
CRP												
26	randomised trials	serious [a]	not serious [b]	not serious	not serious	publication bias strongly suspected [c]	828	750	-	MD **0.01 lower** (0.06 lower to 0.03 higher)	⊕⊕◯◯ LOW	IMPORTANT
Adiponectin												
7	randomised trials	serious [d]	serious [e]	not serious	serious [f]	none	240	266	-	MD **0.29 higher** (0.63 lower to 1.21 higher)	⊕◯◯◯ VERY LOW	IMPORTANT
TNF-a												
8	randomised trials	serious [g]	not serious	not serious	not serious	none	250	232	-	MD **0.05 lower** (0.13 lower to 0.02 higher)	⊕⊕⊕◯ MODERATE	IMPORTANT
IL-6												
13	randomised trials	serious [h]	not serious	not serious	not serious	publication bias strongly suspected [i]	471	435	-	MD **0.02 lower** (0.12 lower to 0.08 higher)	⊕⊕◯◯ LOW	IMPORTANT
ICAM-1												

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	nut consumption	control	Relative (95% CI)	Absolute (95% CI)		
15	randomised trials	not serious [i]	not serious	not serious	not serious	none	539	508	-	MD **0.68 higher** (0.53 lower to 1.89 higher)	⨁⨁⨁⨁ HIGH	IMPORTANT
VCAM-1												
14	randomised trials	not serious [k]	not serious	not serious	not serious	none	419	385	-	MD **2.83 higher** (8.85 lower to 14.51 higher)	⨁⨁⨁⨁ HIGH	IMPORTANT
FMD												
9	randomised trials	not serious [l]	not serious	not serious	not serious	none	326	326	-	MD **0.79 higher** (0.35 higher to 1.23 higher)	⨁⨁⨁⨁ HIGH	IMPORTANT

CI: Confidence interval; **MD:** Mean difference

a. The studies were viewed as being in the category of 'serious limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'high risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'high risk' needed to be categorised as either 'serious limitations' or 'very serious limitations'. In view of the potential implications of the 'high risk' aspects on the quality of the body of evidence, 'serious limitations' was selected

b. I squared value of 20%, indicating minimal heterogeneity

c. Funnel plot indicates likelihood of publication bias

d. The studies were viewed as being in the category of 'serious limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'high risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'high risk' needed to be categorised as either 'serious limitations' or 'very serious limitations'. In view of the potential implications of the 'high risk' aspects on the quality of the body of evidence, 'serious limitations' was selected

e. I squared value of 79% indicating considerable heterogeneity

f. Total sample size is greater than 400, however 95% CIs overlap no effect and include appreciable benefit or harm

g. The studies were viewed as being in the category of 'serious limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'high risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'high risk' needed to be categorised as either 'serious limitations' or 'very serious limitations'. In view of the potential implications of the 'high risk' aspects on the quality of the body of evidence, 'serious limitations' was selected

h. The studies were viewed as being in the category of 'serious limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'high risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'high risk' needed to be categorised as either 'serious limitations' or 'very serious limitations'. In view of the potential implications of the 'high risk' aspects on the quality of the body of evidence, 'serious limitations' was selected

i. Funnel plot indicates likelihood of publication bias

j. The studies were viewed as being in the category of 'no limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'unclear risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'unclear risk' needed to be categorised as either 'no limitations' or 'serious limitations'. In view of the potential implications of the 'unclear risk' aspects on the quality of the body of evidence, 'no limitations' was selected

k. The studies were viewed as being in the category of 'no limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'unclear risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'unclear risk' needed to be categorised as either 'no limitations' or 'serious limitations'. In view of the potential implications of the 'unclear risk' aspects on the quality of the body of evidence, 'no limitations' was selected

l. The studies were viewed as being in the category of 'no limitation'. This category was selected as the risk of bias assessments for each study resulted in mainly 'unclear risk' (see risk of bias assessment charts). In accordance with the GRADE guidelines, 'unclear risk' needed to be categorised as either 'no limitations' or 'serious limitations'. In view of the potential implications of the 'unclear risk' aspects on the quality of the body of evidence, 'no limitations' was selected

Supplementary material 1: PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	2
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5 -6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5 -6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supplementary material 2
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	7

Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	8,9
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7-8

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	8,9
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Supplementary material 8, 9
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Table 2, Figure 2, Figure 3, Supplementary material 6
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Table 2
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Figure 4
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Table 2, Supplementary material 3, 4, 5

DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	24 - 30
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	28 - 30
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	30
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	31

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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