



FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

Systematic Review of the Evidence for a Relationship between Walnuts and Endothelium-dependent Vasodilation

Prepared by: Food Standards Australia New Zealand

Date: October 2014

Executive Summary

<i>Does consumption of walnuts influence endothelium-dependent vasodilation?</i>	
Food-health relationship	Consumption of walnuts is associated with improvements in endothelium-dependent vasodilation
GRADE rating	Unassessable
Component	Notes
<i>Body of evidence</i>	There was no existing meta-analysis or systematic review of the relationship between eating walnuts and endothelium-dependent vasodilation (EDV). Therefore we undertook a new systematic review. Six studies met the inclusion criteria and were used to examine the relationship. There were acute (1) and short-term (5) studies, using two methods of measuring EDV.
<i>Consistency</i>	Three out of five short-term studies claimed an improvement in EDV in terms of a significant difference between the walnut and control groups. The differences are small and, owing to small sample sizes, imprecise. The acute study found an effect in hypercholesterolaemic people only. Study quality, and small sample numbers limit the conclusions that can be drawn with regard to consistency.
<i>Causality</i>	Randomised controlled trials (RCTs) are an appropriate study design for assessing causality. Six crossover RCTs were included in the assessment. However methodological flaws do not allow a conclusion that a causal relationship has been demonstrated.
<i>Plausibility</i>	Several plausible mechanisms have been proposed for the effect of walnut consumption on endothelial function, for example, from their arginine content. However, the proposed mechanisms have not yet been demonstrated in the literature.
<i>Generalisability</i>	The systematic review included studies from the USA and continental Europe, published between 2004 and 2014, so should be generally applicable to Australia and New Zealand. However, most studies included only older, overweight/obese subjects or those with diet-related conditions, such as hypercholesterolaemia or Type 2 diabetes, with baseline flow-mediated dilations that were substantially lower than those observed in healthy people. This may limit generalisability to younger adults or Australian and New Zealand adults without these conditions.

FSANZ has conducted a systematic review on walnut consumption and endothelium-dependent vasodilation (EDV). In doing this review, FSANZ has followed the requirements of the *Application Handbook* and Schedule 6 of Standard 1.2.7 – Nutrition, Health and Related Claims, for the required elements of a systematic review.

Six relevant randomised controlled trials were identified. All of these trials were at high risk of bias, but otherwise met all the inclusion criteria. It is not clear whether the studies were conducted for a sufficient duration to allow any changes to stabilise or whether the occlusion measurements were conducted for a short enough time to make an appropriate measurement. Therefore, FSANZ considers the relationship between walnuts and EDV to be unassessable at this time.

Contents

- Executive Summary i
- 1 Introduction 1
 - 1.1 Food..... 1
 - 1.2 Health effect..... 1
 - 1.2.1 Measurement of endothelium-dependent vasodilation..... 2
 - 1.2.2 Factors that affect FMD and PAT measurements 2
 - 1.2.3 FMD and PAT as measures of CVD risk 3
 - 1.3 Proposed relationship..... 3
- 2 Evaluation of evidence 3
 - 2.1 Methods 4
 - 2.1.1 Search strategy 4
 - 2.1.2 Inclusion and exclusion criteria..... 4
 - 2.1.3 Unpublished material..... 4
 - 2.1.4 Study selection, data extraction..... 5
 - 2.1.5 Statistical analyses..... 5
 - 2.1.6 Sub-group analyses 5
 - 2.2 Results..... 5
 - 2.2.1 Search results 5
 - 2.2.2 Included studies 6
 - 2.2.3 Extracted data..... 8
 - 2.2.4 Quality assessment of individual studies 9
 - 2.2.5 Outcome data 9
 - 2.3 Summary of evidence..... 12
- 3 Weight of evidence..... 12
 - 3.1 Degree of certainty..... 12
 - 3.1.1 Study biases 12
 - 3.1.2 Indirectness..... 13
 - 3.1.3 Imprecision..... 13
 - 3.1.4 Inconsistency 13
 - 3.1.5 Other methodological issues 14
 - 3.2 Assessment of body of evidence..... 15
 - 3.2.1 Consistency and causality of relationship 15
 - 3.2.2 Plausibility 15
 - 3.2.3 Characterisation of the food or property of food..... 16
 - 3.2.4 Summary of the body of evidence 17
 - 3.3 Applicability to Australia and New Zealand..... 17
 - 3.3.1 Intake required for effect 17

3.3.2	Target population	17
3.3.3	Extrapolation from supplements	17
3.3.4	Adverse effects	17
4	Conclusion	18
	Acknowledgement	18
	References.....	19
	Appendix 1: Summary of included studies.....	22
	Appendix 2: Assessment of risk of bias in individual studies.....	28
	Appendix 3: Table of excluded studies	31

1 Introduction

In 2012, the European Union (EU) authorised a claim that ‘walnuts contribute to the improvement of the elasticity of blood vessels’ under Article 13(1) which permits function claims. The condition attached to this claim was a statement that the beneficial effect was obtained with a daily intake of 30 g walnuts (European Commission regulation (EU) No. 432/2012 of 16/05/2012). FSANZ notes that the evidence assessed by the European Food Safety Authority (EFSA), on which this claim was based, examined endothelium-dependent vasodilation (EDV as the outcome) (EFSA 2011). While EFSA’s conclusions were drawn from the scientific literature available at the time, new studies are now available.

FSANZ is considering whether a relationship between walnuts and EDV can be incorporated into Standard 1.2.7 – Nutrition, Health and Related Claims. The purpose of this paper is to systematically review the evidence for this relationship.

1.1 Food

Walnuts are seeds from the walnut tree (genus *Juglans*) of which there are approximately 20 different species, found in different parts of the world (EFSA 2011). The majority of walnuts available for consumption are the variety known as ‘English’ walnuts (AGMRC 2013; Stahmann Farms 2014). Walnuts can be consumed raw or roasted, intact, as ground meal, or as oil, as well as being incorporated into mixed dishes such as cakes. In the context of this food-health relationship, the food under investigation is the edible portion of the walnut, intact or ground, eaten alone or incorporated into other foods, as well as walnut oil.

The nutrient profile of walnuts varies but they are characterised by a high total fat content (around 69 g/100 g in the uncooked nut), of which approximately 75% is polyunsaturated (~ 65% linoleic acid and 10% alpha linolenic acid) (NUTTAB 2010). Walnuts have a protein content of around 14 g/100 g (NUTTAB 2010) of which around 2 g/100 g is the amino acid arginine (Feldman et al. 2002).

1.2 Health effect

Vasodilation refers to the widening of blood vessels. Endothelium-dependent vasodilation occurs when dilation of blood vessels is influenced by the function of the cells that line the blood vessel wall (the endothelial cells). Dilation occurs primarily as a result of nitric oxide (NO) generation from the amino acid L-arginine, which is released by endothelial cells in response to chemical or physical stress, such as the shear stress that results from increased blood flow. The NO produced leads to increased levels of cyclic guanosine monophosphate (cGMP) which, in turn, reduces intracellular calcium levels in the smooth muscle that lies beneath the endothelial layer. This changes muscle tone and allows the artery to dilate, increasing blood flow (Schwartz et al. 2010).

Many researchers consider dysfunction of the vascular endothelium an early adverse change along the pathway to atherosclerosis and EDV as an independent predictor of cardiovascular disease (CVD) risk (Thijssen et al. 2011; Schwartz et al. 2010; Poredos & Jezovnik, 2013; Inaba et al. 2010; Jablonski et al. 2013).

The health effect in this review is vasodilation assessed by a direct measure of blood vessel dilation through changes in vessel diameter or blood volume, either flow-mediated dilation or peripheral arterial tonometry. This is because there do not appear to be established biomarkers of vasodilation and EFSA did not take into account biomarker evidence in assessing this relationship. In addition, this review does not consider endpoints other than

vasodilation, such as arterial stiffness, that could contribute to the claimed effect of 'elasticity of blood vessels'.

1.2.1 Measurement of endothelium-dependent vasodilation

There are a number of ways in which the function of the endothelium can be assessed, although none of these techniques appear to be standardised or to have established clinical or population cut-off levels for function- or risk-assessment (Woo et al. 2014). Some of these methods involve biochemical measures (e.g. levels of intercellular adhesion molecules) (Schwartz et al. 2010) which do not measure change in blood vessel size and thus do not measure vasodilation *per se*.

There are two main, non-invasive methods that are used to measure vasodilation in research studies: flow mediated dilation/dilatation (FMD) and peripheral arterial tonometry (PAT). Both techniques rely on the induction of reactive hyperaemia (RH) by blocking ('occluding') blood flow in the selected artery and then measuring one or more physical parameters that reflect the increase in blood flow and blood vessel size following removal of the occlusion. The techniques differ in the parameter that is measured and the artery investigated (Schwartz et al. 2010). FMD directly measures vasodilation in the larger conduit arteries, usually the brachial artery, through the use of scanning ultrasound measurements of artery diameter and is recorded as percent change in vessel diameter. PAT measures the change in finger volume after occlusion, and attributes this to vasodilation; it therefore measures effects on microcirculation (Poredos & Jezovnik 2013). PAT is usually reported as the reactive hyperaemia index (RHI), which is sometimes expressed on a logarithmic scale (Framingham RHI, or fRHI). Studies by Dhindsa et al. (2008) and Woo et al. (2014) demonstrated that EDV measured using FMD is significantly and positively correlated with the RHI as measured by PAT.

Typical values reported for FMD range from around 5-15% (Schwartz et al. 2010; Dhindsa et al. 2008; Peters et al. 2012). RHI values have been reported in the range 1.4–2.1 (Woo et al. 2014), or around 0.4–0.1.1 when transformed on the natural logarithmic scale (fRHI) (Hamburg et al. 2008; Rubinshtein et al. 2010). In two studies that measured both FMD and PAT in the same subjects, FMD values of 5-9% were associated with RHI values of 1.7-2.0 (Dhindsa et al. 2008; Woo et al. 2014).

Although FMD and PAT are both used to measure vasodilation, these tests do not have the same endpoints and so results from studies using FMD or PAT are considered separately in this review.

1.2.2 Factors that affect FMD and PAT measurements

There are many factors associated with participant and test characteristics that influence FMD and PAT results that need to be considered when interpreting data, including diet (caffeine and alcohol consumption and the fat content of recent meals), recent aerobic or resistance exercise and supplement/medication use (including aspirin). FMD is generally assessed when subjects are fasted and have avoided exercise, caffeine, alcohol, drugs, stimulants, and medications for a consistent period of time (at least 6 hours). Pre-menopausal women should be tested on days 1-7 of the menstrual cycle. Baseline and post-intervention measurements should be made at the same time of day because FMD can be affected by circadian rhythms (Thijssen et al. 2011; Stoner & Sabatier 2012; Schwartz et al. 2010).

Methodological factors that can influence the outcome of an FMD measurement include the position of the cuff that is applied to constrict blood flow, the time post-constriction at which measurements are taken and the conditions of the instruments used to make measurements.

Measurement of FMD requires skilled ultrasound technicians and considerable inter- and intra-operator variability has been detected (Peters et al. 2012; Thijssen et al. 2011; Stoner & Sabatier 2012; Schwartz et al. 2010). A study by Peretz et al. (2007) that compared different FMD techniques concluded that forearm occlusion is preferable to upper arm occlusion, that continuous ultrasound scanning should be used for at least three minutes in healthy adults (longer in those with arterial disease), and that automated computer-based edge detection software should be used. For PAT, methodological factors that may affect results include inadequate blood flow occlusion, poor signal quality and computer error (Hamburg et al. 2008). Skulas-Ray et al. (2011) reported that repeated PAT testing may change the response to hyperaemia, indicating the importance of adequate controls and randomised allocation.

Importantly for this review, Skulas-Ray et al. (2011) reported that it may require at least 8 weeks before FMD or PAT values stabilise following an intervention.

1.2.3 FMD and PAT as measures of CVD risk

In contrast to the wide acceptance that measures of serum cholesterol concentrations are risk factors for coronary heart disease in populations and individuals, neither FMD nor PAT testing appear to form part of the established methods for assessing CVD risk. However, there does appear to be consensus that measuring endothelial function, using either FMD or PAT, is appropriate in population studies as one of several parameters to assess CVD risk. For example, a US study found a significant inverse relationship between the fRHI and several cardiovascular risk factors, including body mass index, total/HDL cholesterol ratio, diabetes, smoking and use of lipid lowering medication, but not blood pressure (Hamburg et al. 2008). Woo et al. (2014) reported that both FMD and RHI were significantly lower in patients with multivessel and complex coronary artery disease and Rubinshtein et al. (2010) reported that fRHI correlated with subsequent adverse cardiac events in a group of outpatients followed for seven years.

1.3 Proposed relationship

The food-health relationship being assessed in this report is that consumption of walnuts or walnut oil is associated with improvements in EDV.

2 Evaluation of evidence

No existing systematic review on this topic was identified at the time this FSANZ review started. Subsequently, Barbour et al. (2014) published a large systematic review on a range of nut types (including walnuts) and a range of CVD outcomes (including endothelial function). Their review assessed literature published up to November 2012 and included three of the five short-term (4–8 weeks) studies included in the FSANZ review (Ros et al. 2004; West et al. 2010; Ma et al. 2010). It did not include two newer short-term studies identified by FSANZ (Katz et al. 2012; Wu et al. 2014).

The research question used for the current review was: in people with or without increased cardiovascular disease risk, does consumption of walnuts or walnut oil, compared with a walnut-free diet, enhance endothelium-dependent vasodilation?

2.1 Methods

2.1.1 Search strategy

A search was conducted in EMBASE and PubMed on 9 January 2014. A total of 94 studies meeting the search terms were identified. The PubMed search was updated on 2 October 2014 and an additional 14 studies identified.

Broad search terms were used due to the relatively small amount of literature on this topic. The following terms were used:

(Walnut* OR Juglan*) AND (endothel* OR arter* OR dilat*)

The WHO International Clinical Trials Registry Platform was also searched to identify potentially unreported or impending clinical trials on walnuts and EDV, from January 2013 to 20 March 2014.

Hand-searching was performed on the reference lists of articles screened at the full-text stage.

2.1.2 Inclusion and exclusion criteria

To be included in the systematic review the trial must have been randomised and included an appropriate control group. Only experimental studies were considered in this review. Parallel, crossover and Latin square design protocols were acceptable. A minimum walnut consumption of 5 grams was established as representing consumption of at least three walnuts or a teaspoon of walnut oil. The comparator was a diet without walnuts. Outcome measures of EDV, measured using either FMD or PAT, were required. Studies where participants had diagnosed cardiac failure or were taking known vasoactive drugs were excluded as these can affect vasodilation. Acute or short-term studies were included. Studies examining mixed nuts were excluded. Table 1 summarises the inclusion and exclusion criteria.

Table 1 *PICOTS criteria for study selection*

Population	Males and females With or without established cardiovascular risk factors Without cardiac failure
Intervention	Walnuts only (whole, ground or oil) at least 5 g per day
Comparator	Diet without walnuts
Outcome	Flow mediated dilation measured using ultrasound or peripheral artery tonometry assessed on the fingers
Time	Single meal or longer
Study design	Randomised controlled trials

2.1.3 Unpublished material

No unpublished material was used in the analysis.

One clinical trial was identified (Record NCT01884363, Walnut consumption, endothelial function and biomarkers) in the WHO International Clinical Trials Registry Platform. This trial will cover 24 adults with established cardiovascular disease or diabetes and assess change

in RHI, assessed using PAT, following 12 weeks consumption of 28 g per day of walnuts. Primary data collection will not be completed until March 2015.

2.1.4 Study selection, data extraction

Database searches and screening on title and abstract was performed by one reviewer. Two reviewers screened articles at the full-text stage, with differences resolved by consensus or consultation with a third reviewer. Data were extracted by one investigator and cross-checked by a second researcher. Trials were assessed for risk of bias according to the Cochrane Handbook (The Cochrane Collaboration 2009), and were collated using Review Manager version 5.3 (RevMan v5.3), the systematic review software developed by The Cochrane Collaboration (The Nordic Cochrane Centre 2014).

2.1.5 Statistical analyses

No meta-analysis was conducted as part of this report, and no statistical tests were conducted. This was primarily due to inconsistencies in the presentation of data and study design of the included studies, with the length of study, measure reported, and mode of reporting differing among studies. Overall, the studies did not provide sufficient information to re-calculate and re-analyse the data.

2.1.6 Sub-group analyses

The following sub-group analyses were established *a priori* but were not considered quantitatively:

- normocholesterolaemics versus hypercholesterolaemics
- high quality studies versus low quality studies
- vasodilation assessed using FMD compared to PAT
- acute studies (≤ 1 day) compared to short-term studies
- studies in healthy people versus those with significant health complications.

2.2 Results

2.2.1 Search results

Electronic literature searching retrieved 109 records. As a result of using broad search terms, 29 were found to be unrelated to the topic (for example, referring to 'walnut sized' tumours). Following screening on title and abstract, 23 records remained; after full text screening, 6 articles met the inclusion and exclusion criteria. No additional articles were identified through hand-searching the reference lists.

After completing the detailed full-text review, six studies were included in the final analysis. The literature screening process is summarised in Figure 1, with reasons for exclusion of studies at full text screening detailed in Appendix 3.

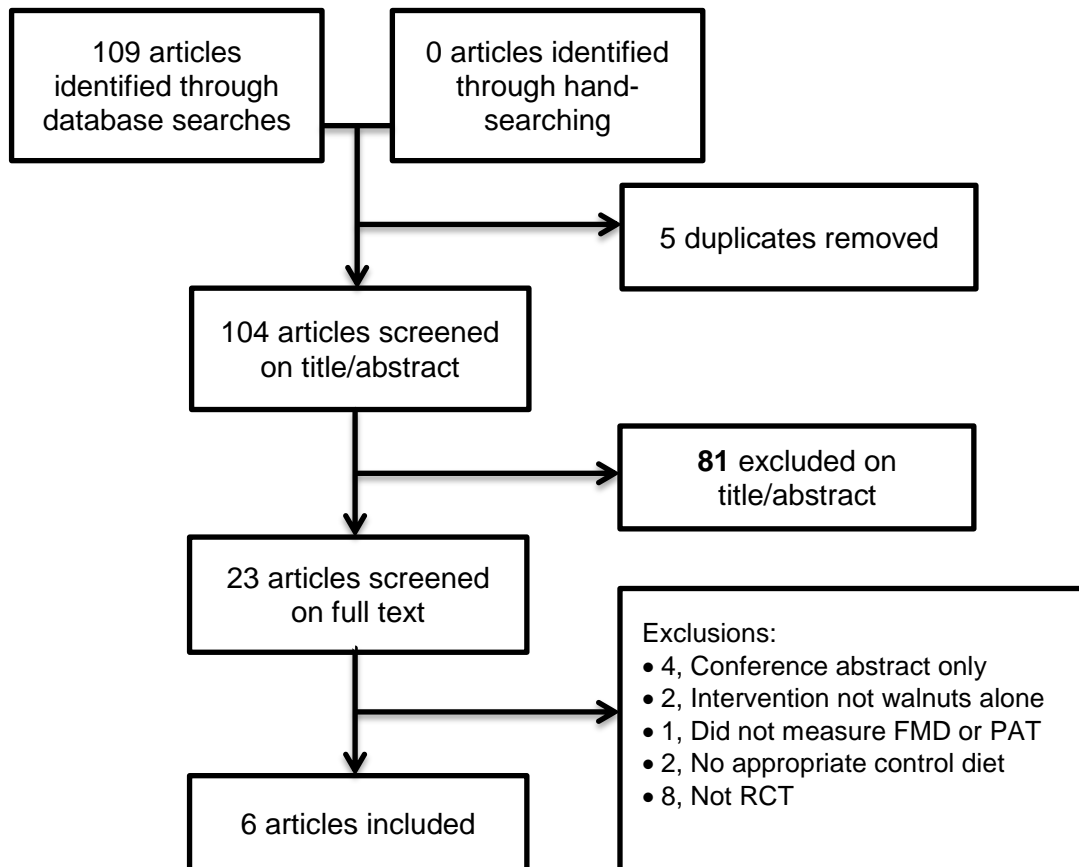


Figure 1 PRISMA diagram of study identification process

2.2.2 Included studies

Trial characteristics are summarised in Table 2 and Appendix 1. Of the six included studies, five were short-term (4–8 weeks) studies. Four of these studies assessed EDV using FMD and one using PAT. The remaining study was an acute (single meal) study where the outcome assessed was FMD.

Three of the included studies were conducted in the USA (Ma et al. 2010; West et al. 2010; Katz et al. 2012) and three were conducted in Europe (Ros et al. 2004; Cortés et al. 2006; Wu et al. 2014). All included studies were funded by the California Walnut Commission.

Table 2 Summary of included studies (all studies are randomised, crossover, single blind controlled trials)

Study	Participants	Intervention	Results (mean ± SD)	Study author/s' conclusions
FMD short-term				
Ros et al. 2004	18 completed Men & women Mean age 55 y HC (mean TC 6.9 mmol/L) BMI not stated	40-65 g walnuts/d 4 wk per arm	<i>End of trial values</i> Control: 3.6 ± 3.3% Walnut: 5.9 ± 3.3% Treatment effect (walnut vs. control) p = 0.043	Walnuts improve endothelium-dependent vasodilation
West et al. 2010	11 completed Men & women Mean age 49 y HC (mean TC 5.8 mmol/L) Mean BMI 28.8 kg/m ²	37 g/day whole walnuts PLUS 15 g/day walnut oil per 10MJ 6 wk per arm	<i>End of trial values</i> Control: 6.1 ± 3.6% Walnut: 6.7 ± 3.3% Treatment effect (walnut vs. control) p = 0.66	Walnuts do not improve endothelium-dependent vasodilation
Ma et al. 2010	22 completed Men and women w/ type 2 diabetes Mean age 58 y Mean TC 4.8 mmol/L (with 54% on lipid lowering medication) Mean BMI 32.5 kg/m ²	56 g walnuts/day 8 wk per arm	<i>Change in FMD</i> Control vs baseline: 1.2 ± 1.6% Walnut vs baseline: 2.2 ± 1.7% p = 0.04 Treatment effect (walnut vs. control) Not given	Walnuts improve endothelium-dependent vasodilation
Katz et al. 2012	40 completed Men and women Mean age 57 All had at least one more risk factor for metabolic syndrome. Mean TC 5.3 mmol/L Mean BMI 33.2 kg/m ²	56 g walnuts/day 8 wk per arm	<i>Change in FMD</i> Control vs baseline: 0.3 ± 1.5% Walnut vs baseline: 1.4 ± 2.4% Treatment effect (walnut vs. control) 1.1% p<0.019	Walnuts improve endothelium-dependent vasodilation
PAT short-term				
Wu et al. 2014	32 completed Healthy men and women Mean age 60 NC or borderline HC (mean TC = 5.7 mmol/L). Normal or overweight BMI (mean 24.9kg/m ²).	43 g walnuts/day 8 wk per arm	fRHI: Control: Δ = 0.13 ± 0.07 Walnut: Δ = -0.06 ± 0.07 Treatment effect (walnut vs control) p = 0.174	Walnuts do not improve endothelium-dependent vasodilation

Study	Participants	Intervention	Results (mean ± SD)	Study author/s' conclusions
FMD acute				
Cortés et al. 2006	24 completed Healthy males and females 12 NC (mean TC 4.8 mmol/L) 12 HC (mean TC 6.5 mmol/L) Normal to overweight BMI	40 g walnuts in single high fat meal	<i>End of trial values</i> FMD NC: Control: 3.9 ± 2.9% Walnut: 4.2 ± 2.3% FMD HC: Control: 2.3 ± 2.2% Walnut: 5.1 ± 1.9% Treatment effect (walnut vs control, NC and HC combined) p = 0.05	Walnuts improve endothelium-dependent vasodilation in HC but not in NC

BMI = body mass index, FMD = flow mediated dilation, fRHI = Framingham reactive hyperaemia index, HC = hypercholesterolaemics, NC = normocholesterolaemics, SD = standard deviation

2.2.3 Extracted data

2.2.3.1 Presentation and estimation of reported values

For the studies reporting FMD, two ways of reporting results were noted; end of trial values, and change in FMD compared to baseline. Both approaches relied on the measurement of artery diameter before and after occlusion. Though researchers are advised to report FMD in absolute (mm) and relative terms (%) (Stoner and Sabatier 2012), only Cortés et al. (2006) attempted to provide this information.

In the first approach, three studies (Ros et al. 2004; West et al. 2010; Cortés et al. 2006) reported results for % FMD for the control and walnut intervention groups separately (mean and standard deviation or standard error) and undertook statistical analyses on these separate values. None of the included studies described the formulas or process that they used to calculate % FMD. FSANZ assumes that they used the following, standard formulas (Harris et al. 2010) to calculate reported %FMD:

$$\%FMD_{\text{baseline}} = [\text{artery diameter post-occlusion}_{\text{prior to intervention}} - \text{artery diameter pre-occlusion}_{\text{prior to intervention}}] / \text{artery diameter pre-occlusion}_{\text{prior to intervention}} * 100$$

$$\%FMD_{\text{control}} = [\text{artery diameter post-occlusion}_{\text{control}} - \text{artery diameter pre-occlusion}_{\text{control}}] / \text{artery diameter pre-occlusion}_{\text{control}} * 100$$

$$\%FMD_{\text{walnut}} = [\text{artery diameter post-occlusion}_{\text{walnut}} - \text{artery diameter pre-occlusion}_{\text{walnut}}] / \text{artery diameter pre-occlusion}_{\text{walnut}} * 100$$

However, although all these three studies provided values for artery diameters pre-occlusion, only Cortés et al. (2006) provided post-occlusion values.

Ma et al. (2010) and Katz et al. (2012) used a different approach. Baseline artery diameters, pre-occlusion, were reported but no further artery values were provided; separate %FMD_{walnut} and %FMD_{control} values were also not provided. The reported results were expressed as

the difference (mean and standard deviation) between %FMD_{intervention (walnut or control)} vs %FMD_{baseline}. The treatment effects were calculated as:

$$\text{Treatment effect walnut (\%)} = \%FMD_{\text{walnut}} - \%FMD_{\text{control}}$$

For the single study using PAT, the RHI was estimated as:

$$\text{RHI} = \left\{ \frac{\text{pulse wave amplitude}_{\text{post-occlusion}}}{\text{pulse wave amplitude}_{\text{pre-occlusion}}} \right\} / \left\{ \frac{\text{pulse wave amplitude}_{\text{non-occluded arm post-occlusion}}}{\text{pulse wave amplitude}_{\text{non-occluded arm pre-occlusion}}} \right\} * (\text{baseline correction factor})$$

The fRHI is the natural logarithm of the RHI but without accounting for the baseline correction factor. fRHI is an index and has no units.

The studies typically report results as percent change from baseline. However this practice can convert a normally-distributed variable into one that is not normally distributed (Vickers, 2001). Some, but not all, authors refer to testing normality or to using non-parametric statistics in the statistical analysis section of their papers.

2.2.4 Quality assessment of individual studies

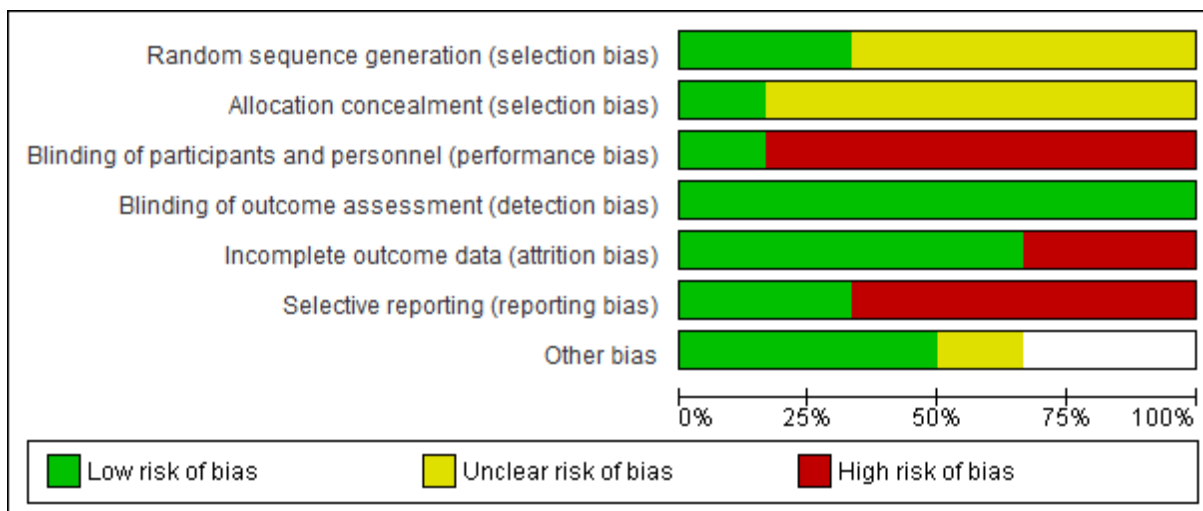
There was a high degree of variability in the quality of included trials (Figure 2). All included studies were considered to have one or more deficiencies or biases. All studies either had an unclear or high risk of bias on at least two of the seven aspects considered. Four of the six studies did not adequately describe randomisation procedures while five studies did not adequately describe allocation concealment. These risks of bias could therefore not be assessed.

The areas where three or more studies had unclear or high risk of bias were randomisation, allocation, and participant blinding. Three of the four short-term FMD studies (Ros et al. 2004, Katz et al. 2012, Ma et al. 2010) were at high risk of bias in reporting outcomes, due to the absence of expected data (i.e. increases in endothelium-dependent vasodilation following consumption of walnuts). Two studies (Wu et al. 2014; West et al. 2010) had a high attrition rate and did not include a comparison of completers vs non-completers.

2.2.5 Outcome data

As only one of the five FMD studies (the acute study, Cortés et al. 2006) provided artery diameters pre- and post-occlusion, and other studies provided only variance data for the chosen method of reporting results, it was not possible to express all results in a common format. For this reason, a meta-analysis covering all short-term FMD studies was not conducted.

Among the four short-term studies that measured FMD, two reported statistically significant improvements in the change in FMD compared to baseline in the walnut groups (Ma et al. 2010; Katz et al. 2012), and one reported a statistically significant improvement in absolute values when compared to control (Ros et al. 2004). None of the studies reported an overall decline in EDV following walnut consumption. However, substantial variance was found within these studies. For example, in the study by Ros et al. (2004), which reported the greatest benefit of walnut consumption, control group FMD (mean and standard deviation) was $3.6 \pm 3.3\%$, whereas in the walnut group it was $5.9 \pm 3.3\%$. Yet, in this study, around half of participants had less than a 2% improvement which emphasises the non-normality of the data.



	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)
Cortes 2006	?	?	-	+	+	+
Katz 2012	?	?	-	+	+	-
Ma 2010	?	?	-	+	+	-
Ros 2004	?	?	-	+	+	-
West 2010	?	?	-	?	-	+
Wu 2014	+	?	-	?	-	-

Figure 2 Risk of bias summary for included trials

Of the four short-term studies measuring FMD, three stated that their trials were powered to detect a difference of 2-3% whereas West et al. (2010) stated that they assumed a difference of 35% in their sample size calculations. FSANZ assumes that the former were describing an

absolute difference in percentage points whereas West et al. (2010) were describing a relative difference. Only the first study conducted (Ros et al. 2004) achieved this difference between the walnut and control groups; all studies published since then have found smaller differences.

The short-term study that assessed EDV using PAT is the most recently published study (Wu et al. 2014). They found no significant differences in the RHI or fRHI between those eating or not eating walnuts. This is the only study that reports a better effect in the control group than the walnut group (although not significant).

In the acute study of FMD (Cortés et al. 2006), %FMD was measured before and after eating walnuts. There were no improvements in %FMD following the walnut meal in normocholesterolaemics. In hypercholesterolaemics, %FMD did improve after the walnut meal, with the increase similar to that observed in the short-term FMD studies. However, in this study, FSANZ reviewers noted some significant inconsistencies in the presented results; no changes were reported in artery diameter before and after occlusion for any group, yet there were differences between groups for %FMD, which is estimated from artery diameters (Cortés et al. 2006).

2.2.5.1 Sub-group analyses

As only one acute FMD study and one PAT study met the inclusion criteria, the *a priori* analyses of study duration and outcome measurement were not able to be conducted. Due to the small number of studies, and the fact that we did not conduct a meta-analysis, we were not able to formally assess the differences between normocholesterolaemics versus hypercholesterolaemics, high quality studies versus low quality studies and studies in healthy people versus those with significant health complications, though these comparisons are discussed in subsequent sections of the report.

2.2.5.2 Participant characteristics

Among the included studies, participants were largely those with one or more CVD risk factors. Studies were conducted among older (generally 50 years and above) and overweight adults, adults with hypercholesterolemia and/or with other conditions such as Type 2 diabetes.

Three of the five short-term FMD studies included hypercholesterolaemic participants (Ros et al. 2004; West et al. 2010; Wu et al. 2014). Participants in Ma et al. (2010) had a mean total cholesterol concentration of 4.9 mmol/L but around half of participants took lipid lowering drugs. Katz et al. (2012) included borderline hypercholesterolaemics (mean total cholesterol 5.3 mmol/L) but participants all had at least one risk factor for metabolic syndrome and were obese (mean body mass index [BMI] 33.3 kg/m²). The acute FMD study included separate analyses of normo- and hypercholesterolaemics and found no effect of walnuts in normocholesterolaemics. The short-term PAT study included primarily borderline hypercholesterolaemic participants (mean total cholesterol concentration 5.7 mmol/L).

Studies differed in other reported CVD-related outcomes assessed. All studies reported baseline blood pressure and/or change in blood pressure, as well as either body weight or BMI. However Ros et al. (2004), while reporting body weight, did not provide information on participants' height that would allow assessment of whether participants were normal weight or overweight. Neither Katz et al. (2012) nor West et al. (2010) reported measures of carbohydrate metabolism. In studies that reported measures related to carbohydrate metabolism, there tended to be no differences between the intervention and control groups. West et al. (2010) and Katz et al. (2012) reported lower blood pressure (systolic and diastolic in West et al, and only systolic in Katz et al. (2012)) in the walnut arm. In contrast, Ma et al.

(2010) reported that both systolic and diastolic blood pressure were lower in the control arm compared to the walnut arm.

2.3 Summary of evidence

Due to a lack of common format of reported data (and additional concerns, that will be outlined in Section 3), a meta-analysis could not be conducted. An observational assessment of the data showed that there was substantial variation among studies. Though three studies reported statistically significant improvements in FMD following walnut consumption, the size of the effect varied greatly and none have replicated the magnitude of effect reported by the first study. Among the five short-term studies, only three lasted eight weeks, which Skulas-Ray et al. (2011) have suggested is the minimum duration necessary to allow FMD measurements to stabilise after a change. Notably, the study with the shortest duration (four weeks) reported the largest effect (Ros et al. 2004).

3 Weight of evidence

3.1 Degree of certainty

Six RCTs were included in this systematic review. Data from these studies were not reported in a common format and were not combined together in a meta-analysis. Exploratory analysis of the studies showed large differences in the effects between studies. Of the five short-term studies, two reported a statistically significant effect of consumption of walnuts compared to control, two reported non-significant differences (one of which favoured the control group) and one did not describe this analysis. The single acute study reported more favourable results in hypercholesterolaemic than normocholesterolaemic people.

3.1.1 Study biases

Other than in the study by West et al. (2010), randomisation and/or allocation procedures were poorly described in all studies, therefore, these procedures were determined as being of unclear risk.

As the intervention in all cases was walnuts and, it was not possible for participants to be blinded to the intervention. While this is unlikely to have affected the results of the acute (single meal) study, it could potentially affect participant behaviour in the short-term studies. All the included studies provided dietary advice to participants, aimed at minimising dietary differences between the two arms of the crossover studies and most also monitored diet during each intervention. It is unclear as to whether lack of participant blinding could have affected the outcome measured, but it is considered to be unlikely, providing the participant did not reveal the information to the outcome assessor while the measurements were being done.

Outcomes assessment was blinded in all studies using FMD; however, this description might not have included accidental unblinding of the assessor by the participant. Wu et al. (2014) did not identify whether or not the outcome assessor was blinded, but the PAT test is less operator-dependent than FMD and was therefore assessed as low risk for this study.

An important deficiency in the majority of studies was the absence, or only partial reporting, of relevant data such as artery diameter before and after occlusion in both test and control groups. Among the FMD studies, only West et al. (2010) and Cortés et al. (2006) provided data for artery diameter before and after occlusion in both groups.

In the FMD studies, the outcome reported was either a percentage or a difference between two percentages. The statistical tests applied by the authors assume normality, however, no information was provided on whether this assumption was met by the data.

Due to the small number of studies and different measurement and outcome reporting techniques used, publication bias was not assessed using a funnel plot or similar technique.

3.1.2 Indirectness

As the small number of included studies were largely conducted in older adults at considerable risk of CVD, for example due to obesity, hypercholesterolemia and/or type 2 diabetes, there is insufficient evidence on which to generalise the findings to the broader adult population in Australia and New Zealand. This concern also applied to the highest quality study (Ros et al. 2004), where mean TC was 6.9 mmol/L, mean participant age was 55 years and, for women, only post-menopausal women were included.

In addition, there is a significant issue regarding the directness of the measurements used. Five out of six papers included in this review measured endothelial function by way of FMD, and one paper assessed EDV using PAT. FMD and PAT measure distinct sets of arteries (large and small, respectively) and whilst two studies (Dhindsa et al. 2008; Woo et al. 2014) have described positive, significant correlations between FMD and PAT, there is generally only a modest relationship between measures of micro- and macrovascular activity (Dhindsa et al. 2008). This raises the question of whether one, both, or neither are accurate and appropriate measures of endothelial function.

Thus, the indirectness is such that the relationship cannot be assessed.

3.1.3 Imprecision

In general, the studies were limited by small participant numbers, which are likely to lead to substantial imprecision in the findings. Across the four short-term FMD studies, participant numbers totalled 111 enrolments and 91 completions, with the largest study, Katz et al. (2012), including 46 enrolments and 40 completions. In the short-term PAT study, numbers were 57 and 32 respectively and in the short-term FMD study there were 24 participants. In total, 192 participants enrolled across all studies and 147 completed the interventions.

Data on confidence intervals were not provided in any of the included studies, only standard deviations or standard errors were presented. In most studies, these values, relative to the reported mean values, were large (see Section 2.2). Thus, it was necessary to down-grade for imprecision.

Due to the small number of studies, different ways of measuring and reporting EDV and the narrow range of walnut quantities studied (43–56 g/day) a dose response analysis was not conducted.

3.1.4 Inconsistency

Among the short-term studies, there was inconsistency in the effect size but not in the direction of effect, which favoured walnuts in four out of five included studies, although this was not always statistically significant. Studies assessing EDV using FMD have not replicated the magnitude of effect reported by the first study. The study which assessed EDV using PAT described a not-significant effect which favoured the control group rather than the walnut group (Wu et al. 2014)

Consideration of the available data, including the single acute study, suggests that effect size may be influenced by the cholesterol status of the participants, but this could not be investigated further. Overall, FSANZ considers that there was some inconsistency across the studies.

3.1.5 Other methodological issues

Although all of the included studies had clearly stated hypotheses, two studies did not feature assessment of endothelial function as a primary study outcome; there was no statistically significant effect of walnuts found in these studies (West et al. 2010; Wu et al. 2014).

With one exception, all short-term studies assessed compliance with diets through use of diet records (or similar assessment) and required participants to provide empty walnut packets to confirm compliance. Although West et al. (2010) was the only study that provided all meals and snacks, they did not comment on whether or not they assessed compliance with the provided diets. Ros et al. (2004) and Wu et al. (2014) were the only studies that assessed compliance through biochemical measurement, assessing serum gamma tocopherol and alpha linolenic acid levels respectively.

Study duration (4–8 weeks in the short-term studies) may be insufficient for changes in EDV to stabilise, noting that Skulas-Ray et al. (2011) consider that a minimum of eight weeks is required for measures to stabilise. The studies with six to eight weeks per arm found a smaller effect than the single study using four weeks per arm.

Many of the studies included in this review reported a change compared to baseline, rather than compared to control. This is misleading, as the aim of a randomised controlled trial is to compare outcomes between groups, who are comparable except for the trial intervention, not within groups (Bland and Altman 2011). Interventions may result in within-group changes between baseline and end-of-intervention measurements, but these changes should not be used as statistical evidence of treatment effects (Bland and Altman 2011).

Among those studies that reported they had considered statistical power, most were likely to be underpowered to detect changes in FMD/PAT that were lower than predicted (e.g. Ma et al. 2010; Katz et al. 2012; West et al. 2010). Wu et al. (2014) did not base their power assessment on the predicted change in EDV and Cortés et al. (2006) did not comment on statistical power. West et al. (2004) estimated the number of participants required for crossover trials to have sufficient power (power = 0.8) to detect a significant effect on FMD. To observe a 10% increase in FMD, as reported in all short-term studies other than Ros et al. (2004), 132 participants would be required. Thus, only Ros et al. (2004) is likely to have been sufficiently powered. As Ros et al. (2004) was the first study, it is possible that subsequent studies were aiming to replicate their results.

As noted earlier, the control group and baseline FMD values varied widely among studies and were relatively low (from 3.4–8.8%) compared to literature reports that indicate values of up to 15% are typical in healthy adults (Schwartz et al, 2010). As FMD is affected by measurement technique as well as participant characteristics, and as measurement techniques were not fully standardised across studies, this raises a question as to the comparability of results between FMD studies. For example, Peretz et al. (2007) recommends that post-occlusion measurements be taken continuously for at least 180 seconds, whereas none of the included studies measured artery diameter for more than 120 seconds (West et al, 2010), and some measured for as little as 50 seconds (Katz et al. 2012; Ma et al. 2010). The variation in timing of recording means that we cannot be sure that all of the included studies measured peak vasodilation, and represent the same measure of EDV. In addition, three of the studies measured FMD by occluding the brachial artery in the upper arm (Katz et al. 2012; Ma et al. 2010; West et al. 2010), while Ros et al. (2004) and Cortés et

al. (2006) occluded the brachial artery in the forearm. Peretz et al. (2007) report that measurements from the forearm yield lower FMD values with better reproducibility.

As both measures are sensitive to small changes in participant or testing characteristics measures should be standardised as much as is feasible. There has been a recent focus on reporting shear stress in conjunction with FMD, and normalising FMD percentages to shear rate, to help control for the heterogeneity of blood flow across participants (Harris et al. 2010) Though collecting and reporting all available measures, including baseline vessel diameters, absolute changes in diameter, and the area under the curve of the shear rate, is highly recommended (Harris et al. 2010), none of the included studies in this review reported the full suite of measures.

3.2 Assessment of body of evidence

3.2.1 Consistency and causality of relationship

All included studies that measured FMD were consistent in their findings of direction of effect of walnuts on EDV. Three of the five showed a significant effect of walnut consumption, although the magnitude of the effect differed. However only two of the studies specifically tested the effect of the walnut phase against the control phase and only one of these reported a significant difference. Therefore we conclude that the other four studies had a non-significant difference between the walnut and control phases. Due to the small number of studies, lack of high quality studies and narrow walnut intake range, a dose response analysis was not conducted. There was a high degree of imprecision among studies, due to the small total number of studies and participants and the high relative standard deviation/standard error. As studies were largely conducted among older adults with existing hypercholesterolaemia, type 2 diabetes, metabolic syndrome and/or obesity, generalisability to the healthy Australian and New Zealand population is not appropriate. Potential bias across included studies was substantial, particularly in relation to randomisation, allocation and outcomes reported.

Though the studies that measured FMD were consistent in their reporting of the direction of effect (i.e. in favour of walnuts) regardless of statistical significance, the study that assessed EDV using PAT did not show a favourable change as a result of walnut consumption (fRHI control: 0.13, walnuts: -0.06; Wu et al. 2014). Given that only one study in this review used PAT to assess EDV, it is difficult to know whether this finding is due to random variation or whether it represents a fundamental discrepancy between the effects of walnuts on FMD and PAT. PAT is a measure of microvascular function and is less well established as a technique than FMD, which measures macrovascular function. Therefore comparisons or concordance between techniques must be viewed with caution.

While crossover, controlled trials are generally suitable for demonstrating whether or not a causal relationship exists between consumption of a food, such as walnuts, and a health outcome, such as EDV, in all studies the control and walnut dietary interventions differed in their fatty acid profile. The fatty acid composition of diets is known to influence serum lipid profiles and could, potentially, also influence EDV. Therefore we cannot be certain that the achieved effects are due specifically to the consumption of walnuts.

3.2.2 Plausibility

Several mechanisms have been proposed to explain how walnuts could plausibly affect EDV. These include the presence of the amino acid arginine and walnuts' high polyunsaturated fatty acid profile.

In relation to arginine, the likely amount consumed per day, assuming 50 g of walnuts were eaten, would be in the order of 1 g/day. It is unclear how much arginine would need to be consumed to lead to the observed outcomes in the included studies although Ros et al. (2004) cite a figure of 2 g/day and above.

In relation to dietary fatty acid profile, the walnut diets delivered a higher intake of polyunsaturated fatty acids (PUFA) than the control diets and it is plausible that this could contribute to some or all of the observed effects. For example, in the study by Ros et al. (2004), those on the walnut diet consumed approximately 7 g per day more PUFA than those on the control diet. In the study by West et al. (2010) there was an additional study arm (not included in this systematic review) where the intervention included flaxseed oil as well as walnuts and walnut oil, which yielded a higher intake of alpha linolenic acid (ALA)¹. In this arm, statistically significant increases in FMD were found, from $6.1 \pm 1.1\%$ in the control arm to $8.2 \pm 1.0\%$ in the flaxseed oil+walnut arm, compared to $6.7 \pm 1.0\%$ in the walnut-only arm. Participants in the flaxseed oil+walnut arm consumed an average of 6.5 g/day ALA whereas those in the walnut-only arm consumed 3.6 g/day (control diet included 0.8 g/day). However, a recent study by Skulas-Ray et al. (2011) found that consumption of 3.4 g/day of long chain omega 3 PUFAs for 8 weeks did not change EDV (measured using PAT) in healthy adults with moderate hypertriglyceridaemia.

The use of walnut oil in West et al. (2010) also raises the issue of whether the active ingredient in walnuts, whatever it may be, would be present in walnut oil, and at what concentration. Future assessments may need to consider this issue when considering the plausibility of the relationship and the food stuffs used in the interventions.

The study by Ros et al. (2004) was designed and assessed as a short-term study of the sustained effects of eating walnuts on EDV. However, the study design also included a test meal, containing walnuts, that was administered four hours before FMD testing, and contained half the daily consumption of walnuts (mean 25 g walnuts). This was similar to the acute study reported by Cortés et al. (2006), where participants ate 40 g walnuts four hours before FMD, although the magnitude of change in FMD observed by Ros et al. (2.2% absolute increase in FMD) was greater than that found by Cortés (0.9% increase). This leaves open the question of whether or not the effect of walnuts on EDV is an acute or short-term effect, or both. The evidence presented in this review is insufficient to answer that question.

3.2.3 Characterisation of the food or property of food

In all included studies, the food was described as being 'walnuts' or 'walnut oil' and further information was not always provided to identify the specific type of walnuts studied (English vs other varieties) or any processing the nuts had undergone. Wu et al. (2014) described the nuts only as being shelled and prepackaged. Katz et al. (2012) and Ma et al. (2010) identified the nuts as being shelled, unroasted English walnuts. Only Ros et al. (2004) provided data on the nutrient composition of the studied nuts and also identified the nuts as being shelled and raw, but did not indicate whether English walnuts were used. Cortés et al. (2006) and West et al. (2010) did not provide information on the walnuts consumed. The study by West et al. (2010) used a combination of walnuts and walnut oil to achieve a set dietary lipid profile. Fitschen et al. (2011) found that black walnuts do not improve EDV after a high fat meal, whereas English walnuts do improve EDV.² This lack of clarity about the food being investigated should be resolved in any further reviews of walnuts, as it could significantly influence the specific food to which the relationship applies.

¹ It is this second arm for which data are cited in the review by Barbour et al. (2014)

² Fitschen et al. (2011) was not included in this review as it did not have an adequate (non-walnut) control group

3.2.4 Summary of the body of evidence

The evidence base consists of a small number of studies and a low overall number of study participants. Most included studies are underpowered in that they detected smaller changes in their outcome than had been assumed in their sample size calculations. There is considerable risk of bias and there are methodological issues, including non-uniformity of the FMD method procedures, which strongly suggest further evidence is required to investigate this hypothesised relationship.

The studies were largely conducted among hypercholesterolaemics, overweight or obese participants, participants at risk of metabolic syndrome or those with established Type 2 diabetes. While all of these conditions are prevalent among Australian and New Zealand adults, there is insufficient evidence on which to generalise the findings to a healthy population.

The food in question, walnuts, is inadequately characterised to allow consideration of whether a relationship exists for all varieties of walnuts and all likely types of processing. It is not certain that the food component that is exerting the observed effect is unique to walnuts, rather than some other property that is also present in other foods, such as flaxseed oil.

For a food-health relationship to be substantiated there has to be a consistency of effect across high quality studies. FSANZ considers that, based on the current evidence, the relationship between walnut consumption and EDV is not able to be assessed.

3.3 Applicability to Australia and New Zealand

3.3.1 Intake required for effect

The amounts of walnuts consumed per day in the included studies ranged from 43 to 56 g/day, which is approximately one-third to one-half of a cup, or two small 30 g 'snack boxes'. Mean per capita nut consumption (excluding nuts consumed as ingredients in foods such as cakes and nut butters) was 6 g/day among Australian adults, as reported in the Australian Health Survey (ABS 2014). Among adult nut eaters, median nut consumption was 27 g/day. This suggests a walnut intake consistent with that of the included studies could be achieved. Walnuts are particularly versatile, and can be incorporated into salads and other savoury dishes, sweets, cereals/mueslis and can be eaten raw/roasted as a snack. If future studies determine that any beneficial effects derived from walnuts are also derived from walnut oil, this would offer additional avenues for walnut products to be incorporated into the usual Australian/New Zealand diet.

3.3.2 Target population

As noted earlier, the evidence base is largely derived from studies among older participants with elevated CVD risk. Participants had baseline FMD values considerably lower than those previously described in healthy people. Additional studies are needed to determine if the effect is only present in a sub-group of the population.

3.3.3 Extrapolation from supplements

None of the included evidence related to dietary supplements.

3.3.4 Adverse effects

No adverse effects were reported in any of the included studies.

4 Conclusion

Based on the body of evidence, including the small number of studies and participants, methodological and reporting issues, and data that are almost exclusively in people with CVD risk factors, it was not possible to assess the relationship between walnut consumption and EDV.

Acknowledgement

We acknowledge permission from the Cochrane Collaboration to use the Review Manager 5.3 software.

References

- Agricultural Marketing Resource Centre (AGMRC) (2013) English walnuts. Downloaded from http://www.agmrc.org/commodities_products/nuts/english-walnuts-profile/ on 2 October 2014
- Australian Bureau of Statistics (ABS) (2014) 43640DO005_20112012 Australian Health Survey: Nutrition First Results – Foods and Nutrients, 2011–12 — Australia. Downloaded from <http://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/4364.0.55.0072011-12?OpenDocument> on 3 October 2014
- Barbour JA, Howe PR, Buckley JD, Bryan J, Coates AM (2014) Nut consumption for vascular health and cognitive function. *Nutrition Research Reviews* 27(1):131-58
- Bland JM and Altman DG (2011) Comparisons against baseline within randomised groups are often used and can be highly misleading. *Trials* 12:264
- Cortés B, Núñez I, Cofán M, Gilabert R, Pérez-Heras A, Casals E, Deulofeu R, Ros E (2006) Acute effects of high-fat meals enriched with walnuts or olive oil on postprandial endothelial function. *Journal of the American College of Cardiology* 48(8):1666-71
- Dhindsa M, Sommerlad SM, DeVan AE, Barnes JN, Sugawara J, Ley O, Tanaka H (2008) Interrelationships among noninvasive measures of postischemic macro- and microvascular reactivity. *Journal of Applied Physiology* 105:427-432
- EFSA (2011) Scientific Opinion on the substantiation of health claims related to walnuts and maintenance of normal blood LDL-cholesterol concentrations (ID 1156, 1158) and improvement of endothelium-dependent vasodilation (ID 1155, 1157) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA Journal* 9(4):2074. Available at <http://www.efsa.europa.eu/en/efsajournal/doc/2074.pdf>
- Feldman EB (2002) The scientific evidence for a beneficial health relationship between walnuts and coronary heart disease. *Journal of Nutrition* 132(5):1062S-1101S
- Fitschen PJ, Rolfhus KR, Winfrey MR, Allen BK, Manzy M, Maher MA (2011) Cardiovascular effects of consumption of black versus English walnuts. *Journal of Medicinal Food* 14(9):890-8
- Hamburg NM, Keyes MJ, Larson MG, Vasan RS, Schnabel R, Pryde M, Mitchell GF, Sheffy J, Vita JA, Benjamin EJ (2008) Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham heart study. *Circulation* 117:2467-2474
- Harris RA, Nishiyama SK, Wray DW, Richardson RS (2010) Ultrasound Assessment of Flow-Mediated Dilatation. *Hypertension* 55:1075-1085
- Inaba Y, Chen JA, Bergmann SR (2010) Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. *International Journal of Cardiovascular Imaging* 26(6):631-40
- Jablonski KL, Racine ML, Geolfos CJ, Gates PE, Chonchol M, McQueen MB, Seals DR (2013) Dietary sodium restriction reverses vascular endothelial dysfunction in middle-aged/older adults with moderately elevated systolic blood pressure. *Journal of the American College of Cardiology* 61(3):335-43

- Katz DL, Davidhi A, Ma Y, Kavak Y, Bifulco L, Njike VY (2012) Effects of walnuts on endothelial function in overweight adults with visceral obesity: a randomized, controlled, crossover trial. *Journal of the American College of Nutrition* 31(6):415-23
- Ma Y, Njike VY, Millet J, Dutta S, Doughty K, Treu JA, Katz DL (2010) Effects of walnut consumption on endothelial function in type 2 diabetic subjects: a randomized controlled crossover trial. *Diabetes Care* 33(2):227-32
- NUTTAB (2010) Nutrient tables for use in Australia. Food Standards Australia New Zealand
- Peretz A, Leotta D, Sullivan J, Trenga C, Sands F, Aulet M, Paun M, Gill E, Kaufman J (2007) Flow mediated dilation of the brachial artery: an investigation of methods requiring further standardization. *BMC Cardiovascular Disorders* 7:11
- Peters SA, den Ruijter HM, Bots ML. (2012) The incremental value of brachial flow-mediated dilation measurements in risk stratification for incident cardiovascular events: a systematic review. *Annals of Medicine* 44(4):305-12
- Poredos P, Jezovnik MK (2013) Testing endothelial function and its clinical relevance. *Journal of Atherosclerosis and Thrombosis* 20(1):1-8
- Ros E, Núñez I, Pérez-Heras A, Serra M, Gilabert R, Casals E, Deulofeu R (2004) A walnut diet improves endothelial function in hypercholesterolemic subjects: a randomized crossover trial. *Circulation* 109(13):1609-14
- Rubinshtein R, Kuvin JT, Soffler M, Lennon RJ, Lavi S, Nelson RE, Pumper GM, Lerman LO, Lerman A (2010) Assessment of endothelial function by non-invasive peripheral arterial tonometry predicts late cardiovascular adverse events. *European Heart Journal* 31(9):1142-8
- Schwartz BG, Economides C, Mayeda GS, Burstein S, Kloner RA (2010) The endothelial cell in health and disease: its function, dysfunction, measurement and therapy. *International Journal of Impotence Research* 22(2):77-90
- Skulas-Ray AC, Kris-Etherton PM, Harris WS, Vanden Heuvel JP, Wagner PR, West SG (2011) Dose-response effects of omega-3 fatty acids on triglycerides, inflammation and endothelial function in healthy persons with moderate hypertriglyceridemia. *American Journal of Clinical Nutrition* 93:243-252
- Stahmann Farms Pty Ltd (2014) All about walnuts. <http://www.stahmannfarms.com.au/all-about-nuts/walnuts/> Accessed 2 October 2014
- Stoner L, Sabatier MJ (2012) Use of ultrasound for non-invasive assessment of flow-mediated dilation. *Journal of Atherosclerosis and Thrombosis* 19(5):407-21
- The Cochrane Collaboration (2009) *Cochrane Handbook for Systematic Reviews of Interventions*. Higgins, JPT and Green, S (eds) Version 5.0.2
- The Nordic Cochrane Centre, The Cochrane Collaboration. Review Manager (RevMan) Version 5.3. 2014. Copenhagen
- Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, Parker B, Widlansky ME, Tschakovsky ME, Green DJ (2011) Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *American Journal of Physiology. Heart and Circulatory Physiology* 300(1):H2-12

Vickers AJ (2001) The use of percentage change from baseline as an outcome in a controlled trial is statistically inefficient: a simulation study. *BMC Medical Research Methodology* 1:6 doi:10.1186/1471-2288-1-6

West SG, Wagner P, Schoemer SL, Hecker KD, Hurston KL, Likos Krick A, Boseska L, Ulbrecht J, Hinderliter AL (2004) Biological correlates of day-to-day variation in flow mediated dilation in individuals with Type 2 diabetes: a study of test-retest reliability. *Diabetologia* 47:1625-31

West SG, Krick AL, Klein LC, Zhao G, Wojtowicz TF, McGuinness M, Bagshaw DM, Wagner P, Ceballos RM, Holub BJ, Kris-Etherton PM (2010) Effects of diets high in walnuts and flax oil on hemodynamic responses to stress and vascular endothelial function. *Journal of the American College of Nutrition* 29(6):595-603

Woo JS, Jang WS, Kim HS, Lee JH, Choi EY, Kim JB, Kim WS, Kim KS, Kim W (2014) Comparison of peripheral arterial tonometry and flow-mediated vasodilation for assessment of the severity and complexity of coronary artery disease. *Coronary Artery Disease* 25(5):421-6

Wu L, Piotrowski K, Rau T, Waldmann E, Broedl UC, Demmelmair H, Koletzko B, Stark RG, Nagel JM, Mantzoros CS, Parhofer KG (2014) Walnut-enriched diet reduces fasting non-HDL-cholesterol and apolipoprotein B in healthy Caucasian subjects: a randomized controlled cross-over clinical trial. *Metabolism* 63(3):382-91

Appendix 1: Summary of included studies

Table 1a Study details of the included studies on endothelium-dependent vasodilation assessed using flow mediated dilation of the brachial artery – short-term studies

Study	Objective Study design Funding source	Participant characteristics Sample size Attrition	Intervention Amount consumed Duration Control diet	Methods for health effect measurement	Study quality Confounders Power	Results	Conclusion Adverse effects
Ros et al. 2004	<p><i>Objective:</i> To determine if eating walnuts improves EF in HC subjects</p> <p><i>Study design:</i> Randomised, controlled, crossover trial</p> <p><i>Funding:</i> California Walnut Commission, Spanish Ministry of Health</p>	<p><i>Participants:</i> Spanish men and women (25-75 years men; post menopause – 75 years women; mean age 55 years).</p> <p>Healthy, NS, HC (mean TC 6.9 mmol/L) BMI not stated</p> <p>Not taking lipid or hormone medication or nutrient supplements</p> <p><i>Sample size & attrition</i> 21 enrolled 20 completed (8 male) 18 underwent FMD assessment</p>	<p><i>Intervention:</i> Walnuts with Mediterranean diet</p> <p>Specific walnut type not identified.</p> <p><i>Amount:</i> 40-65 g/day in proportion to energy intake, replacing isoenergetic amount of olive oil (control). Based on reported mean E, mean walnut consumption not stated but estimated at ~ 50 g/d.</p> <p><i>Duration:</i> 4 weeks each arm. No washout; 4 week run in of dietary adherence</p> <p><i>Control diet:</i> Isoenergetic, Mediterranean diet incl olive oil. Total fat averaged 33% E in</p>	<p>FMD: non-fasting, 4 hours after ingestion of a standardised low fat lunch incorporating half daily allowance of walnuts or olive oil.</p> <p>4.5 minute blood flow occlusion forearm; post-occlusion measurements averaged across 60-90 seconds post deflation, measured at end diastole.</p> <p>Statistical analysis was repeated measures ANOVA with order and treatment as independent variables. Two tailed t test for paired samples.</p> <p><i>Compliance:</i> Serum gamma tocopherol levels used as a biomarker of compliance with walnut diet. Overall dietary compliance assessed</p>	<p><i>Quality:</i> Background diet not controlled except via provision of dietary advice.</p> <p>Unclear whether the effect measured is an acute or chronic one as a walnut meal was administered 4 hours before FMD testing, supplying half the daily amount.</p> <p>No test for normality</p> <p><i>Confounders:</i> Walnut diet decreased TC and LDL-C cf control diet</p> <p>Fatty acid composition of walnut & control diets differed</p> <p><i>Power:</i></p>	<p>Artery diameter (pre- and post-occlusion; mean ± SD): Baseline: Pre: 4.6±0.8 mm Post: not provided Control: Pre: 4.7±0.7 mm Post: not provided Walnut: Pre: 4.7±0.7 mm Post: not provided</p> <p>Mean FMD (± SD): Baseline: 3.4±3.7% Control: 3.6±3.3% Walnut: 5.9±3.3% p=0.043</p> <p>Only 9/18 participants demonstrated a >2% increase in FMD; 3/18 had minor increases, 3/18 change in FMD, 3/18 declined</p>	<p>A statistically significant effect of walnut consumption on FMD was observed.</p> <p>Study quality rated as moderate-high.</p> <p>No adverse effects noted. Walnuts reported to be well tolerated</p>

Study	Objective Study design Funding source	Participant characteristics Sample size Attrition	Intervention Amount consumed Duration Control diet	Methods for health effect measurement	Study quality Confounders Power	Results	Conclusion Adverse effects
			both groups but PUFA was higher and MUFA lower in walnut diet.	using 7 day diet recalls.	Likely to be sufficiently powered due to large observed effect. Statistical analysis by paired t-test		
Ma et al. 2010	<p><i>Objective:</i> To determine effect of daily walnut consumption on EF, CVD biomarkers & anthropometry in T2DM</p> <p><i>Design:</i> Randomised, controlled, crossover trial</p> <p><i>Funding:</i> California Walnut Commission</p>	<p><i>Participants:</i> Adults with T2DM 30-75 years, overweight or obese (mean BMI 32.5 kg/m²), NS, 42% male, USA Mean age 58 y</p> <p>Excluded if on vasoactive or blood pressure lowering medication, or had known atherosclerosis</p> <p>Mean TC 4.8 mmol/L with 54% on lipid lowering medication</p> <p><i>Sample size & attrition:</i> 24 enrolled, 22 completed</p>	<p><i>Intervention:</i> Shelled, unroasted, English walnuts,</p> <p><i>Amount:</i> 56 g/day</p> <p><i>Duration:</i> Each arm 8 weeks; 8 week washout, 4 week run in</p> <p><i>Control diet:</i> Background diet in walnut and control arms was ad libitum, isoenergetic diet</p>	<p>FMD: fasting (minimum 8 hours). 5 minute blood flow occlusion on upper arm; post-occlusion measurements averaged across 60 seconds post deflation.</p> <p>Paired t tests used to compare baseline mean values for all outcome measures by group. Intention to treat analysis</p> <p><i>Compliance:</i> Assessed by checking walnut bags. 3 day diet records completed in each phase</p>	<p><i>Quality:</i> Background diet not controlled except via provision of dietary advice</p> <p>No test for normality</p> <p><i>Confounders:</i> Fatty acid composition of walnut & control diets differed</p> <p>Blood pressure lower in the control group than the walnut group.</p> <p>No test for normality</p> <p><i>Power:</i> Likely to be underpowered due to lower than predicted FMD difference</p>	<p>Artery diameter (pre- and post-occlusion): Baseline: 4.2±0.8 mm Post#: 4.6 mm Walnut & control: values not provided</p> <p>Mean FMD: Baseline: 8.6±4.3%, Control: 9.8%# Walnut: 10.8%#</p> <p>Mean absolute difference FMD: Control vs baseline: 1.2±1.6%, p<0.05 Walnut vs baseline: 2.2±1.7%, p<0.05 Walnut vs control: +1.0%#, p=0.04</p>	<p>A statistically significant increase in FMD seen in the walnut arm compared to the control arm</p> <p>Study quality rated as moderate</p> <p>No adverse effects noted although the control diet lowered blood pressure compared to the walnut diet</p>

Study	Objective Study design Funding source	Participant characteristics Sample size Attrition	Intervention Amount consumed Duration Control diet	Methods for health effect measurement	Study quality Confounders Power	Results	Conclusion Adverse effects
West et al. 2010	<p><i>Objective:</i> To examine the effects of ALA on cardiovascular responses to acute stress, flow-mediated dilation of the brachial artery and blood concentrations of endotheelin-1 and arginine-vasopressin</p> <p><i>Design:</i> Randomised, controlled, crossover trial</p> <p><i>Funding:</i> California Walnut Commission</p>	<p><i>Participants:</i> Male and female adults with unmedicated HC. Mean age 49.3±1.7 years, mean BMI 28.8 kg/m²</p> <p>NS, proportion male not stated, USA</p> <p>Excluded if using medication for HC, hypertension, inflammatory disease. Excluded if history of CVD, hypertension, diabetes or other systemic disease.</p> <p>Mean TC 5.8 mmol/L</p> <p><i>Sample size & attrition:</i> 12 enrolled and randomised, one subject excluded due to mild arrhythmia at rest. Final n = 11</p>	<p><i>Intervention:</i> Walnuts and walnut oil</p> <p><i>Amount:</i> 37 g/day whole walnuts PLUS 15 g/day walnut oil per 10MJ</p> <p><i>Duration:</i> Each arm 6 weeks; no washout.</p> <p><i>Control diet:</i> “Average American” diet, providing similar amounts of total fat/CHO, protein, cholesterol and nutrients.</p> <p>During intervention, walnuts replaced major food sources of protein (meat and full fat dairy).</p> <p>All food provided for both diets. Walnuts and oils were used in baked goods, salad dressings, pesto, and half the daily walnuts were consumed as a snack.</p>	<p>FMD: 5 minute blood flow occlusion on forearm; post-occlusion measurements peak diameter taken as largest average diameter in the 2 min deflation sequence, typically observed 40-70 seconds post-deflation.</p> <p>FMD measured as the percentage change in arterial diameter after hyperaemia.</p> <p>Treatment effects were assessed using mixed models, using intention to treat analysis. Tukey post hoc tests were used.</p> <p><i>Compliance:</i> All food provided. No other measure of compliance</p>	<p><i>Quality:</i> Background diet controlled.</p> <p>Data tested for normality and transformed where appropriate.</p> <p><i>Confounders:</i> The addition of walnuts to the diet increased the fibre content of diet.</p> <p>Additional trial arm (not included in this systematic review) used walnuts and flax oil.</p> <p><i>Power:</i> Powered to detect a 35% change in FMD.</p>	<p>Artery diameter (pre- and post-occlusion): Baseline: Not provided Control: Pre: 4.3±0.7 mm Post (peak): 4.5±0.7 mm Walnut: Pre: 4.3±0.7 mm Post (peak): 4.6±0.7 mm</p> <p>Mean percent change in FMD: Baseline: Not recorded Control: 6.1±3.6% Walnut: 6.7±3.3% Walnut vs control, p = 0.66</p>	<p>No change in FMD seen in the walnut arm compared to the control arm</p> <p>Study quality rated as moderate</p> <p>No adverse effects.</p> <p>Additional trial arm (not included in this systematic review) using walnuts and flax oil described a significant increase in % change in %FMD.</p>

Study	Objective Study design Funding source	Participant characteristics Sample size Attrition	Intervention Amount consumed Duration Control diet	Methods for health effect measurement	Study quality Confounders Power	Results	Conclusion Adverse effects
Katz et al. 2012	<p><i>Objective</i> To investigate the effects of daily walnut consumption on EF and other biomarkers of cardiac risk in a population of overweight individuals with visceral adiposity</p> <p><i>Design</i> Randomised, controlled, crossover trial</p> <p><i>Funding:</i> California Walnut Commission</p>	<p><i>Participants:</i> NS adults (30-75 years, mean age 57 yrs) with BMI>25 kg/m² and waist circumference >100 cm (men) or 87 cm (women). All had at least one more risk factor for metabolic syndrome. TC mean 5.3 mmol/L</p> <p>BMI 25-35 kg/m². (mean 33.2). 40% male. USA</p> <p>Excluded if diagnosed atherosclerosis, diabetes or other major illnesses. Excluded if using NSAIDs or vasoactive, lipid lowering or antihypertensive medications for <3 mths. Proportion taking lipid lowering drugs not stated</p> <p><i>Sample size and attrition:</i> 46 randomised, 40 completed FMD</p>	<p><i>Intervention:</i> Whole, unroasted English walnuts</p> <p><i>Amount:</i> 56 g/d</p> <p><i>Duration:</i> Each arm 8 weeks. 4 week run in and washout</p> <p><i>Control diet:</i> Background diet was ad libitum diet without walnuts.</p>	<p>FMD: minimum 8 hour overnight fast. 5 min occlusion. Artery diameter reported between 50-80 s post deflation.</p> <p>Statistical analysis was repeated measures ANOVA, with multivariate ANOVA models to assess combined effects of independent variables (age, race, BMI, hypertension, dyslipidemia, treatment sequence) using intention-to-treat analysis.</p> <p><i>Compliance:</i> Assessed by 3 day food diaries and walnut consumption log sheets</p>	<p><i>Quality:</i> Background diet not controlled except via provision of dietary advice</p> <p>No test for normality</p> <p><i>Confounders:</i> Fatty acid composition of walnut & control diets differed (higher PUFA during walnut arm).</p> <p>BMI (p=0.016) and body weight (p=0.019) reduced during the control arm compared to the walnut arm.</p> <p>16% attrition</p> <p><i>Power:</i> Likely to be underpowered due to lower than predicted FMD difference</p>	<p>Artery diameter (pre- and post-occlusion): Not provided</p> <p>Mean FMD: Baseline: 8.8±2.4% Control: 9.1%# Walnut: 10.2%#</p> <p>Mean difference FMD: Control vs baseline: 0.3±1.5% Walnut vs baseline: 1.4±2.4% (p<0.05) Walnut vs control: 1.1% (95% CI = 0.2, 2.0) p=0.019</p>	<p>Consumption of walnuts led to a greater FMD response than a walnut free diet</p> <p>Study quality rated as moderate</p>

Table 1b Study details of the included studies on endothelium-dependent vasodilation assessed using peripheral artery tonometry (PAT) – short-term study

Study	Objective Study design Funding source	Participants Sample size	Intervention	Methods	Study quality	Results	Conclusion Adverse effects
Wu et al. 2014	<p><i>Objective:</i> To assess effect of walnuts on a range of parameters. Primary outcome was non-HDL cholesterol; EF was secondary outcome</p> <p><i>Design:</i> Randomised, controlled, crossover trial</p> <p><i>Funding:</i> California Walnut Commission</p>	<p><i>Participants:</i> Healthy adults (mean age 60 years), 25% male, all female participants were post-menopausal. NC or borderline HC (mean TC = 5.7 mmol/L). Normal or overweight BMI (mean = 24.9kg/m²). Germany (Caucasians)</p> <p>Excluded if taking medicines to control diabetes, lipid levels, blood pressure, inflammation or hormone levels</p> <p><i>Sample size & attrition:</i> 57 randomised (using SAS and a block design), 40 completed full trial but only 32 underwent peripheral artery tonometry.</p>	<p><i>Intervention:</i> Shelled whole walnuts</p> <p><i>Amount:</i> 43 g shelled whole walnuts to replace 30 g saturated fat</p> <p><i>Duration:</i> of each arm was 8 weeks; 2 week run-in and washout</p> <p><i>Control diet:</i> Nut free, ad libitum, isoenergetic western type diet (35% total fat, 15% sat fat)</p>	<p>Participants fasted overnight and were given a high fat meal (72% E) 4 hours before PAT measurement</p> <p>Statistical analysis used a mixed model to adjust for gender, age, BMI, diet sequence and repeated measures.</p> <p><i>Compliance:</i> Assessed by checking walnut bags and plasma levels of ALA. Overall dietary patterns monitored with 4 day diet records during each phase</p>	<p><i>Quality:</i> Lack of information on attrition to PAT testing and consideration of the implications of this</p> <p>No test for normality</p> <p><i>Confounders:</i> Baseline diets were not strictly controlled and walnut diet contained more energy from fat (39.2%) than the control diet (32.7%), lower saturates and higher polyunsaturates.</p> <p>Baseline RHI and fRHI results suggest the control and diet arms were not comparable but no statistical test carried out to show this.</p> <p>43% attrition</p> <p><i>Power:</i> Power calculation not based on predicted change in RHI or fRHI</p>	<p>RHI (mean ±SEM) Control: Baseline 1.92±0.10, End control diet: Δ = 0.05±0.08</p> <p>Walnut: Baseline 2.09±0.10. End walnut diet: Δ = -0.07±0.10</p> <p>p=0.724 (walnut vs control, adjusting for age, gender, BMI & diet sequence)</p> <p>Final RHI values not presented by authors</p> <p>fRHI: Control: Baseline 0.42±0.06, Δ = 0.13±0.07</p> <p>Walnut: Baseline 0.51±0.06, Δ = -0.06±0.07</p> <p>p=0.174 (walnut vs control, adjusting for age, gender, BMI & diet sequence)</p> <p>Final fRHI values not presented by authors</p>	<p>No significant differences in PAT seen between walnut & test group</p> <p>Study quality rated as moderate</p> <p>No adverse effects noted</p>

Table 1c Study details of the included studies on endothelium-dependent vasodilation assessed with three techniques in acute studies

Study	Objective Study design Funding source	Participants Sample size	Intervention	Methods	Study quality	Results	Conclusion Adverse effects
Cortés et al. 2006	<p><i>Objective:</i> To assess if walnuts added to a fatty meal have different effects on a range of parameters, including EF, compared to response to olive oil</p> <p><i>Design:</i> Acute study (single meal), crossover</p> <p><i>Funding:</i> California Walnut Commission</p>	<p><i>Participants:</i> Healthy NS, normal to overweight BMI, NT. 83% men</p> <p>Excluded if taking medication or antioxidant supplements</p> <p><i>Sample size & attrition:</i> 24 total (20 male): 12 NC (mean TC 4.8 mmol/L) 12 HC (mean TC 6.5 mmol/L)</p> <p>Report did not identify if there were any withdrawals (assumed no dropouts due to short study)</p>	<p><i>Intervention:</i> Single high fat test meal (63%E) with or without walnuts, administered in a controlled environment.</p> <p><i>Amount:</i> 40 g walnuts in high fat meal</p> <p><i>Duration:</i> Single meal 1 week period between test control and walnut phases</p> <p><i>Control diet:</i> Control meal was the same but walnuts were replaced with 25 mL olive oil.</p> <p>Both groups followed a cholesterol lowering Mediterranean diet</p>	<p>FMD of brachial artery assessed before and 4 hrs after high fat test meal. Supervised between test meal and FMD</p> <p>4.5 min cuff occlusion</p> <p>Statistical analysis by repeated measures ANOVA with 3 factors (NC vs HC, olive vs walnut, baseline vs postprandial)</p> <p><i>Compliance:</i> Background diet compliance assessed via 7 day food record.</p>	<p><i>Quality:</i> No test for normality</p> <p>p values not provided separately for NC vs HC ppts</p> <p>Mean values for artery diameter & %FMD do not align for all groups (e.g. same artery diameters before & after control meal reported as different %FMD values)</p> <p><i>Confounders:</i> Control diet used likely to give greater decline in FMD than a standard meal with lower fat content</p> <p><i>Power:</i> No power calculation provided.</p>	<p>Artery diameter (pre- and post-meal)</p> <p><i>Control:</i> NC: Pre 4.5±0.8 mm Post: 4.5±0.8 mm HC: Pre 4.9±0.5 mm Post 4.9±0.5 mm</p> <p><i>Walnut:</i> NC: Pre 4.5±0.8 mm Post 4.5±0.8 mm HC: Pre 4.8±0.5 mm, Post 4.8±0.5 mm</p> <p>FMD (mean ± SE)</p> <p><i>Control meal</i> NC: Pre 4.7±1.4% Post 3.9±2.9% HC: Pre 3.6±1.3% Post 2.3±2.2%</p> <p><i>Walnut meal</i> NC: Pre 4.2±1.4% Post 4.2±2.3% HC: Pre 4.1±1.9% Post 5.1±1.9%</p> <p>p=0.05 walnut vs olive oil</p> <p>No BP changes noted (systolic or diastolic)</p>	<p>For NC, meal type did not affect FMD response. For HC, FMD was greater after walnut meal than after the olive oil meal.</p> <p>Study quality rated as moderate</p> <p>No adverse effects noted</p>

FSANZ estimated from presented data

ALA = alpha linolenic acid; BMI = body mass index; CVD = cardiovascular disease; E = energy intake from the diet; EF = endothelial function; FMD = flow mediated dilation of the brachial artery; fRHI = Framingham reactive hyperaemia index; HC= hypercholesterolaemics; MUFA = monounsaturated fats; NC = Normocholesterolaemics; NS = Nonsmokers; RBC = red blood cell; RHI = Reactive hyperaemia index; PUFA = polyunsaturated fats; SAS = statistical software from the SAS Corporation; T2DM = Type 2 diabetes

Appendix 2: Assessment of risk of bias in individual studies

Ros et al. (2004)

Bias	Review authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear	Not specifically addressed in the published paper, but states that participants were randomly allocated to dietary intervention
Allocation concealment (selection bias)	Unclear	Not specifically addressed in the published paper
Blinding of participants and personnel (performance bias)	High risk	No placebo. Participants knew they were receiving the intervention.
Blinding of outcome assessment (detection bias)	Low risk	Outcome assessors were blinded; authors report intra-observer reproducibility for a separate study
Incomplete outcome data (attrition bias)	Low risk	3/21 participants did not complete study
Selective reporting (reporting bias)	High risk	Post occlusion artery diameters not reported

Ma et al. (2010)

Bias	Review authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear	Not specifically addressed in the published paper
Allocation concealment (selection bias)	Unclear	Not specifically addressed in the published paper
Blinding of participants and personnel (performance bias)	High risk	No placebo. Participants knew they were receiving the intervention.
Blinding of outcome assessment (detection bias)	Low risk	Outcome assessors were blinded.
Incomplete outcome data (attrition bias)	Low risk	2/22 participants did not complete study
Selective reporting (reporting bias)	High risk	Values for artery diameter and FMD% for control and walnut not provided; baseline FMD not reported separately for each arm

West et al. 2010

Bias	Review authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear	Not specifically addressed in the published paper
Allocation concealment (selection bias)	Unclear	Not specifically addressed in the published paper
Blinding of participants and personnel (performance bias)	High risk	Participants were not blinded due to the use of whole nuts. Providers were blinded.
Blinding of outcome assessment (detection bias)	Unclear risk	Not stated
Incomplete outcome data (attrition bias)	High risk	Low proportion (11/20) completed FMD as equipment only became available part way through study; no sub-analysis to show the characteristics of this group
Selective reporting (reporting bias)	Low risk	All anticipated outcomes were measured although baseline FMD was not presented separately for control vs intervention

Katz et al. 2012

Bias	Review authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear	Not specifically addressed in the published paper
Allocation concealment (selection bias)	Unclear	Not specifically addressed in the published paper
Blinding of participants and personnel (performance bias)	High risk	No placebo. Participants knew they were receiving the intervention.
Blinding of outcome assessment (detection bias)	Low risk	Outcome assessors were blinded.
Incomplete outcome data (attrition bias)	Low risk	8/40 participants did not complete study
Selective reporting (reporting bias)	High risk	Values for artery diameter and FMD% for control and walnut not provided; baseline FMD not reported separately for each intervention

Wu et al. 2014

Bias	Review authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomised using SAS and a block design
Allocation concealment (selection bias)	Unclear	Not specifically addressed in the published paper
Blinding of participants and personnel (performance bias)	High risk	No placebo. Participants knew they were receiving the intervention.
Blinding of outcome assessment (detection bias)	Unclear	Not specifically addressed in the published paper
Incomplete outcome data (attrition bias)	High risk	Only 32/57 participants underwent PAT; no analysis of their characteristics presented
Selective reporting (reporting bias)	High risk	Only baseline and change in RHI or fRHI values presented, and not the post-intervention RHI or fRHI

Cortés et al. 2006

Bias	Review authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear	Not specifically addressed in the published paper
Allocation concealment (selection bias)	Unclear	Not specifically addressed in the published paper
Blinding of participants and personnel (performance bias)	High risk	No placebo. Participants knew they were receiving the intervention.
Blinding of outcome assessment (detection bias)	Low risk	Outcome assessor was blinded to treatment assignment
Incomplete outcome data (attrition bias)	Low risk	No attrition during study
Selective reporting (reporting bias)	Low risk	All anticipated outcomes were measured.

Appendix 3: Table of excluded studies

Study ID	Reason for exclusion
Anon. 2010 Walnuts and arteries, Harvard Heart letter: from Harvard Medical School, 20(9): 6	Review article
Berryman CE, Grieger JA, West SG, Chen CY, Blumberg JB, Rothblat GH, Sankaranarayanan S, Kris-Etherton P. 2013. Acute consumption of walnuts and walnut components differentially affect postprandial lipemia, endothelial function, oxidative stress, and cholesterol efflux in humans with mild hypercholesterolemia. Journal of Nutrition, 143: 788-794 (Interventions were whole walnuts, walnut skins, defatted nutmeat and walnut oil only)	No appropriate control
Bhardwaj R; Manivannan S; Gharib W; Warden B; Hobbs G; Jain A. 2012. Acute effects of diets rich in almonds and walnuts on endothelial function in humans. Circulation, 136, 21 SUPPL. 1	Conference abstract
Brzezinski A. 2007 [Review], Gastroenterology and Hepatology, 3(10): 787-788	Review article
Duttaroy AK. 2003, Therapy and clinical trials, Current Opinion in Lipidology, 14(4): 397-399	Review article
Einecke D. 2003. Contribution of healthy nutrition. Coronary disease prevention with fish fillet and walnut oil, MMW Fortschritte der Medizin, 145(4): 10	Review article
Esposito K; Ciotola M; Giugliano F; De SM; Giugliano G; D'Armiento M; Giugliano D. 2006, Mediterranean diet improves erectile function in subjects with the metabolic syndrome. International Journal of Impotence Research, 18(4): 405-410	Intervention not restricted to walnuts
Fitschen PJ, Rolfhus K, Winfrey M. Allen B, Manzy M, Maher M. 2011. Cardiovascular Effects of Consumption of Black Versus English Walnuts. Journal of Medicinal Food, 14(9): 890-898 (Interventions were either English walnuts or black walnuts only)	No appropriate control
Hackman R; Yim S; Djurica D; Shindel A; Holt R; Keen C. 2013 Effects of walnut consumption on select vascular biomarkers in hypercholesterolemic postmenopausal women Annals of Nutrition and Metabolism, 63: 1643	Conference abstract
Lopez-Uriarte et al. 2010. Effect of nut consumption on oxidative stress and the endothelial function in metabolic syndrome, Clinical Nutrition, 29, 373-380	Intervention not restricted to walnuts

Study ID	Reason for exclusion
Mirkin G. 1993 Walnuts and serum lipids, The New England Journal of Medicine, 329(5): 358-360	Letter to editor
Mogadam M. 1993 Walnuts and serum lipids, The New England Journal of Medicine, 329(5): 358-360	Letter to editor
Nash SD; Nash D. 2008. Nuts as part of a healthy cardiovascular diet, Current Atherosclerosis Reports, 10(6): 529-535	Review article
Pieters M; Oosthuizen W; Jerling JC; Loots DT; Mukuddem-Petersen J; Hanekom SM. 2005 Clustering of haemostatic variables and the effect of high cashew and walnut diets on these variables in metabolic syndrome patients, Blood Coagulation and Fibrinolysis, 16(6): 429-437	Health outcome not FMD or PAT
Prineas RJ; Kushi LH; Folsom AR; Bostick RM; Wu Y; 1993 Walnuts and serum lipids, The New England Journal of Medicine, 329(5): 359-360	Conference abstract
Vargova V; Mechirova V; Fedacko J; Ryber R; Pella D; Wilczynska A; de MF; Singh RB. 2011. Can nuts consumption modulate cardiovascular diseases? Report of a case and review of literature, Open Nutraceuticals Journal, 4: 88-96	Review article
Yim SJ; Djurica D; Holt RR; Keen CL; Hackman RM. 2013 Effects of walnuts on vascular function and platelet reactivity in postmenopausal women with hypercholesterolemia, FASEB Journal, 27	Conference abstract